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STUDIES IN THE PHYLOGENY OF THE BETULACEAE II. EXTREMES IN THE RANGE OF VARIATION OF FLORAL AND INFLORESCENCE MORPHOLOGY[1]

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(WITH ONE HUNDRED FIGURES)

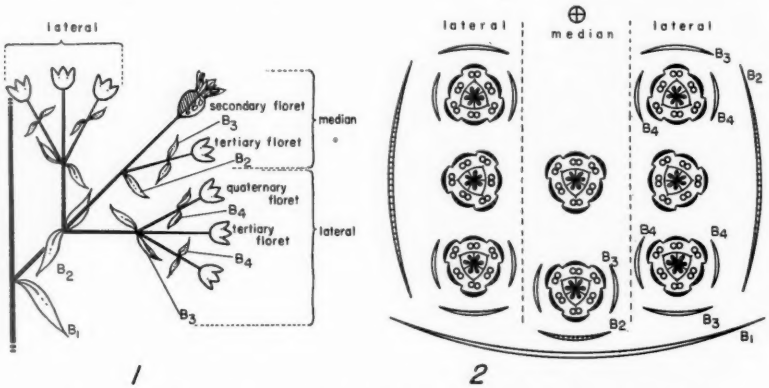
Introduction

In the course of examining the material which forms the basis of the preceding paper of this series (1), a number of variants from the usual floral conditions were noted. Certain of these variations were described at that time, in so far as they served to clarify the more usual morphological conditions in the cymules and florets. The following report extends this survey of the floret and inflorescence morphology of the Betulaceae. Practically all of the variants, some of them frequently, occurred in normal aments. Thus they are to be considered as extremes in the possible range of normal variation rather than as teratological forms.

The variations involve either the individual florets, or the cymules, or both. They can further be classed as representing either extreme reduction or as the development of organs usually more completely suppressed.

[1] The observations here reported were made in part while the writer was privileged to utilize the research facilities of the Biological Laboratories, Harvard University, and of the Botany Department, Cornell University. To the authorities in charge at these institutions, the writer takes this occasion to express his thanks. The project was completed with the aid of technical assistance provided through a research grant from the Graduate School of the University of Minnesota.

The terminology used in the following descriptions is based on that established previously (1, p. 3) and is indicated diagrammatically in figures 1 and 2. In the axil of the primary bract occurs the secondary axis, bearing two sets of secondary bracts and terminating in a secondary floret. In the axils of each of the secondary bracts occur tertiary axes, each bearing a single set of tertiary bracts and terminating in tertiary florets. There is no evidence of further branching of the tertiary axis of the median system. But in the axils



FIGS. 1, 2.—Fig. 1, diagrammatic sketch of single hypothetical cymule to illustrate terminology employed. Fig. 2, floral diagram of same cymule to show inter-relationship of parts.

of the tertiary bracts of the lateral systems occur quaternary axes bearing quaternary bracts and terminating in quaternary florets.

In the following pages reference is made to the transition region from twig to ament. In some genera this region, which is immediately below the ament proper, often exhibits interesting and significant deviations from the morphological conditions in the ament. It is a region in which the internodes are progressively shorter, the leaves smaller, and the buds sometimes replaced by floral structures.

The state of union of the members of the perigon is referred to as syntepaly, a term parallel to sympetaly, synsepaly, etc. Absence of tepals is referred to as atepaly, and independence of tepals as apotepaly. The methods used are essentially those which have already been described in detail (1, p. 2).

Floral variations

1. Tricarpellate pistils

Mention has been made of the occurrence of tricarpellate pistils in *Betula luminifera* (**1**, p. 55); they have also been found by the writer in *Alnus hirsuta*, *A. rubra*, *Betula glandulosa*, *B. grossa*, *B. lutea*, *B. schmidtii*, *Ostryopsis davidiana*, and *O. nobilis*. Zimmermann (**31**) noted a floret with a tricarpellary pistil in the transition region of an ament in *Alnus glutinosa*.

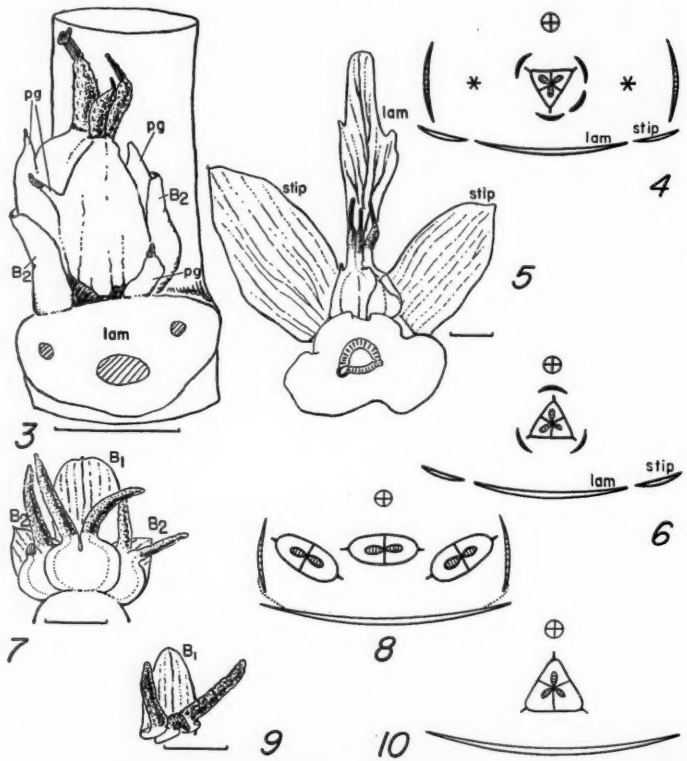
Trimerous pistils may occur in any of the three general subdivisions of the ament; namely, in the much crowded proximal and distal regions, in the main body of the ament, or in the transition region from twig to ament proper. In all the cases observed, the third carpel appeared in florets of otherwise normal aments, except in *B. luminifera* where it was associated with insect injury.

The presence of a third carpel in the ovaries of cymules from the terminal region of the ament was noted only in *B. lutea* (figs. 9, 10). The extra carpel is adaxial here, as it mostly is when there is only a single floret in the axil of a foliar structure. The effect on the position of the extra carpel in the central portion of the ament has not been determined in *Betula* and *Alnus*, because of the extremely flattened condition of the florets.

In *Ostryopsis* the tricarpellary condition was noted in both *O. nobilis* and *O. davidiana*. The same plan obtains in both species, so that the case illustrated for *O. nobilis* (figs. 11–14) may be taken as representative (for a habit sketch see **1**, fig. 99). The tricarpellary floret occurs in the central portion of the characteristically loose ament. The three dorsal bundles (*dr*) of the pistil (fig. 12) arise in essentially the planes in which the bundles to the secondary bract (B_2) and tertiary bracts (B_3) depart from the vascular cylinder of the secondary pedicellar axis (fig. 11). Thus the extra carpel is median to the secondary bract (fig. 13). Trimery does not extend into the perigon. The orientation of the carpels in this material is superficially difficult to interpret because of the twisting and lateral flattening of the floret above the pedicel (fig. 13).

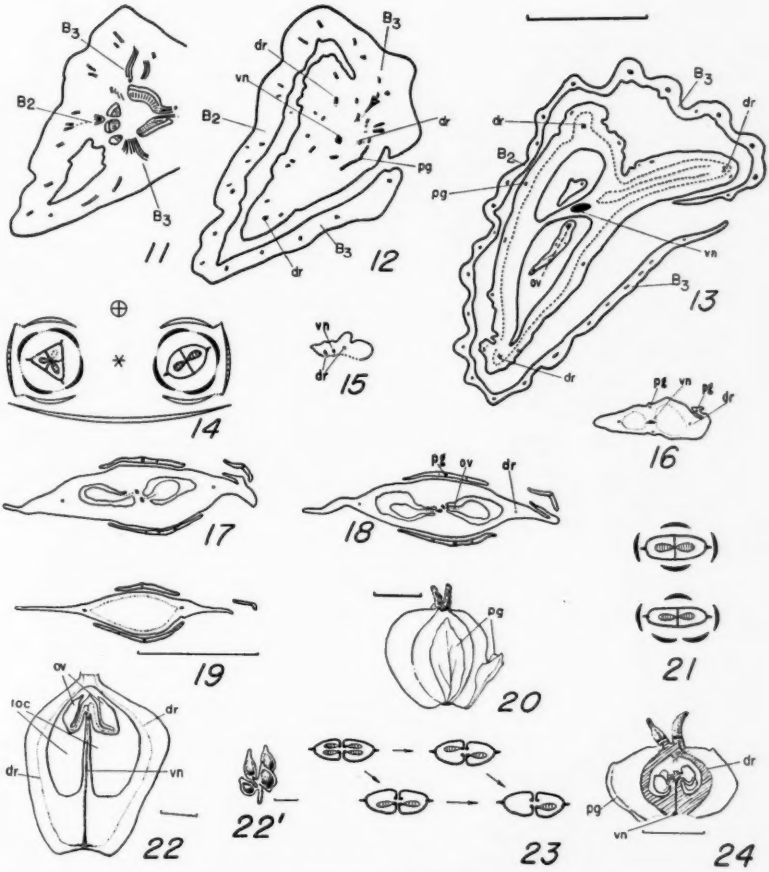
Correlated with this flattening is the lack of development of an ovule and loculus in the third carpel (the adaxial carpel). The two

functional carpels are those which are represented in the usual bicarpellary ovary of this genus (compare the right and left ovaries in the floral diagram, fig. 14). This lends further support to the working hypothesis that the bicarpellary ovary of the Betulaceae originated from a tricarpellary form through the suppression of



FIGS. 3–10.*—Habit sketches and floral diagrams respectively. Figs. 3, 4, *Betula schmidtii*, tricarpellary pistillate floret in axil of reduced foliage leaf. Figs. 5, 6, *B. grossa*, tricarpellary pistillate floret in axil of reduced foliage leaf. Figs. 7–10, *B. lutea*, pistillate; figs. 7, 8, usual type of cymule (young); figs. 9, 10, tricarpellary floret solitary in axil of bract from terminal portion of ament.

* Scale of figures in each case indicated by a line representing 1 mm. Habit sketches and microscopic sections are all camera lucida drawings. Reconstructions of vascular systems are longitudinallyexaggerated to bring out behavior of bundles to better advantage. Transverse portions of fig. 37 is to scale on a 45° projection. Abbreviations same as those used earlier (1).



FIGS. 11–24.—Figs. 11–14, *Ostryopsis nobilis*, pistillate; figs. 11–13, successive transverse sections of single tricarpellary floret with associated bracts; fig. 14, floral diagram of cymule from which sections 11–13 were made. Figs. 15–21, *Alnus rubra*, pistillate; figs. 15–19, successive transverse sections of four-ovuled floret with three tepals present; fig. 20, habit sketch of floret of type shown in figs. 15–19; fig. 21, floral diagram to show two possible types of reconstruction. Fig. 22, *A. japonica*, longitudinal section of floret. Fig. 22′, *A. firma* var. *hirtella*, dissection to show the four ovules. Fig. 23, diagrams to show possible transitions from four- to one-ovuled condition in Betulaceae. Fig. 24, *Betula pumila*, longitudinal section of four-ovuled floret from base of ament.

either the adaxial or median carpel—in *Ostryopsis*, the adaxial carpel. In *Carpinus japonica*, PRANTL (**19**) found that three-styled pistils (presumably also three-carpelled) were of frequent occurrence. Three-parted fruits have been described for *Corylus* by MASTERS (**14**) and figured by WORSDELL (**28**), both cases being interpreted by GUILLAMIN (**12**) as representing a tricarpellary pistil. In the same category occur the three-loculed ovaries of *Corylus* which SPACH (**23**) mentions.

Tricarpellary pistils are oftenest found in the axils of the much reduced foliage leaves of the transition region from twig to ament. Two examples are illustrated, one of *Betula schmidtii* (figs. 3, 4) and the other of *B. grossa* (figs. 5, 6). In general the extra carpel is abaxial if secondary bracts are present (figs. 3, 4) and adaxial if secondary bracts are absent (figs. 5, 6, 9, 10), although exceptions occur.

2. PERIGON UNUSUALLY REDUCED OR WELL DEVELOPED

Reference was made earlier (**1**) to evidence for the presence of a rudimentary perigon in the pistillate florets of many of the Betuleae. As was pointed out at that time (p. 60), four lines of evidence support this concept: the developmental, the anatomical, the presence of glands at the base of the stylar column, and the rare occurrence of foliose tepals.

The best examples of foliose tepals in the pistillate florets of the Betuleae were found in a single collection of *Alnus rubra* (fig. 20). A large proportion of the florets had two or three tepals of the type shown in figure 20. The tepals are usually fused with the ovary wall only near the base. When they occur against the flat side of the ovary their shape is lanceolate to oblanceolate, the margins sparingly toothed. The teeth evidently were originally gland-tipped, although in most cases the glands had fallen off in drying (this being herbarium material). The tepals which occur opposite the margins of the flattened ovaries are often dwarfed and crumpled, as if distorted by restrictions of space within the ament. A summary of the various conditions observed is represented diagrammatically in figure 21, the perigon being either tetramerous or pentamerous. The presence of both tetramerous and pentamerous florets in the same species and even in the same cymule is by no means infrequent in the staminate

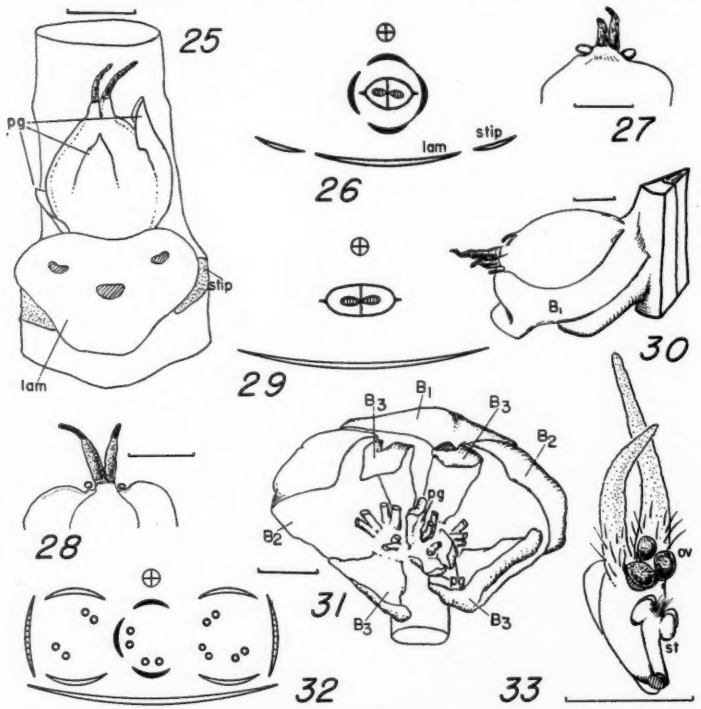
aments of *Alnus*, and it is therefore not surprising to find such tendencies in the pistillate aments. Serial sections of one of these pistillate florets (figs. 15–19) show the distinct nature of the vascular supply to the tepals. The material was mature at the time of collection, so that a well defined, heavily staining abscission layer had formed at the base of the floret. As a result the precise details of the origin of the supply to the tepals were not determined, although it is clear that the bundles to the tepals do not become independent until after the ventral and the dorsal bundles of the carpels depart from the pedicellar vascular supply.

ZIMMERMANN reports (**30**) and figures (**31**) pistillate florets with tetramerous perigons in each of which the tepals are arranged as they are in the staminate florets. These florets were found in the region intermediate between the staminate and pistillate portions of a bisexual ament of *Alnus glutinosa*.

Tepals similar to those occurring in the material of *A. rubra* just described are to be found in florets occurring in the axils of reduced foliage leaves in the transition region in *Betula medwediewii* (*pg*, figs. 25, 26), *B. schmidtii* (*pg*, figs. 3, 4), *B. pumila*, and *B. lutea*. Figure 25, illustrating the condition in *B. medwediewii*, strikingly shows the variable behavior of the tepals, from complete freedom on the one side to fusion for half their length on the other side of the floret. This suggests that recrudescence of the foliose tepal in pistillate florets of the Betuleae does not necessarily provide reliable evidence as to whether the ovary was formerly superior or inferior.

The transition from the rarely foliose tepal in the pistillate florets of the Betuleae to the distinctive glands near the base of the style is easily understood, since similar glands occur on the margins of the tepals in the florets of *Alnus rubra* previously described, as well as regularly on the margins of the tepals in the staminate florets of many species of *Alnus* and *Betula*. So usual is the reduction from foliose tepal to gland within the same ament, or even within the same cymule in staminate florets of *Betula*, that the locus occupied by a conspicuous gland in one floret is clearly recognizable as that occupied by a foliose tepal in another nearby floret. Thus in *B. maximowicziana*, a staminate cymule was chosen in which the adaxial tepal (**1**, fig. 219, *x*) of the secondary floret is represented

only by a characteristic gland. Had another cymule been chosen for illustration from the same ament, tepal *x* might well have been foliose, since many of that type were observed in that species. More



FIGS. 25–33.—Figs. 25, 26, *Betula medwediewii*, pistillate floret with tepals in axil of reduced foliage leaf from base of ament, habit sketch and floral diagram respectively. Fig. 27, *Alnus tenuifolia*, habit sketch of upper part of floret to show glands. Fig. 28, same, of *Betula maximowicziana*. Figs. 29, 30, *B. globispica*, tricarpellary floret with glands, solitary in axil of primary bract, from base of ament, floral diagram and habit sketch respectively. Figs. 31, 32, *Corylus vilmorinii*, teratological staminate cymule with tertiary bracts and tepals present, habit sketch and floral diagram respectively. Fig. 33, *Betula luminifera*, teratological bisexual floret with extruded ovules.

reduced species, such as *B. pendula* (**1**, fig. 198) or *B. lenta* (**1**, fig. 181), have a larger proportion of the tepals of the individual florets represented by glands only. Of considerable interest from this point of view is PAYER'S (**18**) report of four tepal primordia for each

staminate floret in *Betula "alba,"* although not all of these primordia develop into tepals.

In pistillate florets these glands, which represent the apex of a tepal otherwise fused to the ovary wall, vary in number, shape, and constancy of occurrence from species to species, and sometimes may possibly be of taxonomic value. Rarely there are four, as sometimes in *Alnus subcordata* (**1**, fig. 41); more commonly there are two of varying form in the plane of the dorsal bundles, as in *A. sibirica*, *A. incana*, *A. maritima*, *A. spaethii*, *A. tenuifolia* (fig. 27), *A. nepalensis*, *Betula globispica* (fig. 30), *B. coerulea-grandis* (**1**, fig. 98′), *B. utilis* var. *prattii*, and *B. maximowicziana* (fig. 28). Vestigial bundles, whose course suggests that they are remnants of a perigon supply, occur in *Alnus incana*, *A. subcordata*, and *Betula pumila* (fig. 24).

Foliose tepals, when they occur in the pistillate florets, are usually well vascularized. On the other hand, in the tepals of the staminate florets of the Betuleae, a vascular supply may be absent or be represented only by the terminal fragment of a trace disconnected from the main vascular system of the cymule. A corresponding behavior may characterize the much reduced adaxial tertiary bracts sometimes present in the pistillate cymules of *Alnus*. The occasional lack of continuity of the vascular system of a much reduced foliose organ with that of the rest of the cymule suggests an arrested stage in the normal development of most foliar organs—a condition associated, perhaps, with the small *absolute* size attained by these organs when they stop growth. In fact, they are so small at times that they survive without a vascular system. The objections raised by ARBER (**2**) concerning the phylogenetic significance of vestigial traces may perhaps be met on the basis of an analysis of the material concerned in terms of absolute size. It is physiologically unlikely that relatively large organs would persist without a vascular system. Yet relatively small organs, such as the rudimentary glumes of the Gramineae cited by ARBER and the vestigial tepals and bracts of the Betulaceae, are conceivably capable of survival without a vascular system, and of course upon their complete loss cannot leave vestigial bundles behind them. Let the absolute size of the flowering structure as a whole be greater and the organs involved be correspondingly larger, then non-functional but existent organs may be expected in many cases

still to have vascular systems, and since space restrictions are less, to leave vestiges of these vascular systems to survive their disappearance.

In the staminate cymules of the Coryleae, whose florets are usually atepalous, it is only in *Corylus* that tepals have been found in the writer's material. These occur with relative frequency near the base of the ament in otherwise normal *C. americana* (**1**, figs. 253, 254) and *C. maxima* (**1**, figs. 255, 256), the tepals represented being either the adaxial or the abaxial ones of the secondary floret. As pointed out previously (**1**), the key to the interpretation of these as tepals lies in their vascular supply, which is intimately associated with that of the corresponding stamen in the same fashion as that to be found in homologous structures in the staminate florets of *Alnus* and *Betula*. WEISS (**26**) found two tepals to be associated with a dimerous secondary staminate floret in a bisexual cymule from an androgynous ament in "hazel."

A situation which is associated with a disturbance of the aments sometimes occurs in staminate cymules of *Corylus vilmorinii*. These cymules are notable because of the full development of the perigon of the secondary floret and the occasional presence of tepals in the tertiary florets, as well as for the presence of a full complement of tertiary bracts. In the example illustrated (figs. 31, 32) the three tepals of the secondary floret were associated with two stamens (four half-stamens). In other cymules as many as five tepals and three stamens were present in the secondary floret, while sometimes the tertiary florets had a single tepal each and three stamens. In spite of the fact that the material of *C. vilmorinii* is teratological, the phenomena observed fit so well into the basic pattern of the betulaceous floret and cymule that it must be considered as a significant expression of the genic potentialities of the group.

The absence of tepals usually present has been noted in *Alnus glutinosa* in the staminate florets in several instances, especially in florets from the transition region.

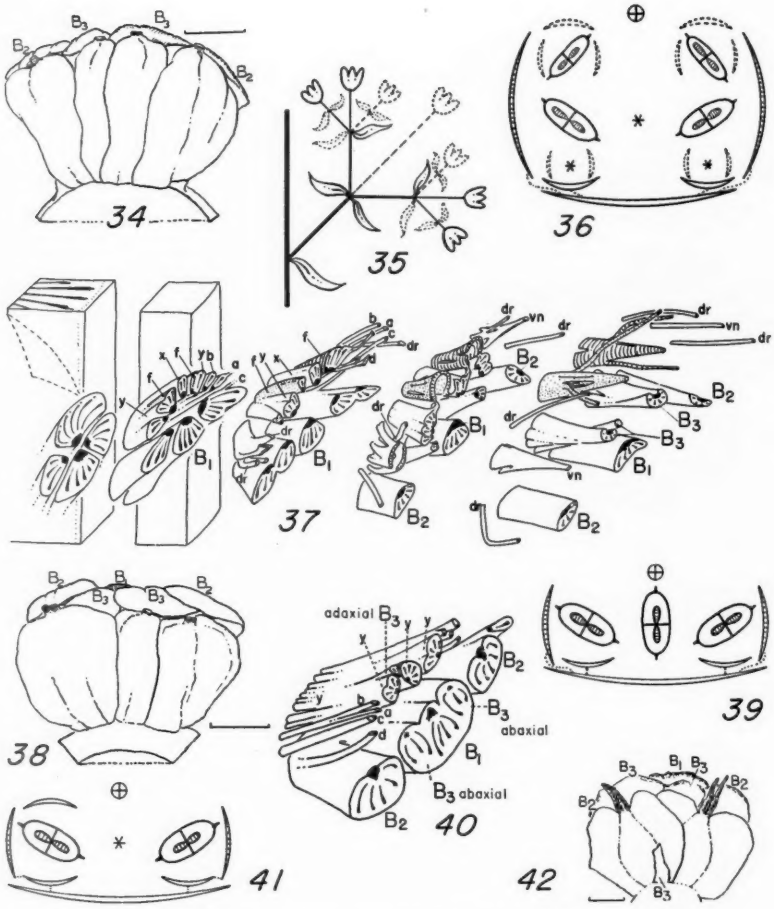
3. VARIATIONS IN OVULE NUMBER

The number of ovules usually ascribed to the ovary in the Betulaceae is two, as shown for *Alnus japonica* (fig. 22). The two loculi

(*loc*), which are poorly defined, usually become continuous through disappearance of the septum above the insertion of the ovules. The disappearance of the septum is associated with the failure of the ventral bundle (*vn*) to extend beyond the insertion of the ovules (*ov*). Sometimes the union of the two loculi occurs below the insertion of the ovules, so that parietal placentation is newly attained. Basically the placentation is axile, since the ventral bundle exists as a single compound structure in most species of the family. Furthermore, tricarpellary ovaries in the family never have parietal placentation, but are consistently axile.

Occasionally in *Alnus firma* var. *hirtella*, *A. rubra*, and *Betula pumila*, more than two ovules occurred. Four ovules were present rather frequently in *Alnus firma* var. *hirtella*, there being one ovule for each margin of the two carpels (fig. 22′). A similar situation was noted by WOLPERT (**28**) in *A. alnobetula*. In the material of *A. rubra* already described, in connection with the presence of a foliose perigon, four or even five ovules are frequently present, the vascularization of a four-ovuled floret being shown in figures 15–19. In figure 17 is shown the departure of traces to ovules on diagonally opposite margins of the two adjacent carpels, while in figure 18 the ovules of the other two margins are being vascularized. The traces to the lower pair of ovules become distinct at a relatively low level in the ovary (fig. 16) and continue parallel to the ventral bundle (*vn*) for an appreciable distance upward while it splits preparatory to supplying the upper pair of ovules. There is no vestigial vascular material extending beyond the insertion of the upper pair of ovules (fig. 19). PAUCHET (**17**) reports three ovules, two in one carpel and one in the other, as sometimes present in the ovary of *Corylus avellana*, not an unexpected finding since BAILLON (**6**) had noted that the anlagen of four ovules are to be found in the pistils of *Corylus* although but rarely did he find all four completely developed. In *Betula pendula* there may sometimes be two ovules to a carpel, according to STREICHER (**24**).

The same general condition as described for *A. rubra* also occurs in the floret of *Betula pumila*, shown split longitudinally in figure 24. This is rather uncommon in *Betula*, the floret illustrated being from the transition region at the base of the ament.



FIGS. 34–42.—Figs. 34–39, *Alnus subcordata*, pistillate; figs. 34–37, four-flowered cymule from central portion of ament, habit, diagram of inflorescence, floral diagram, and reconstruction of vascular system respectively; figs. 38, 39, three-flowered cymule, habit and floral diagram respectively. Fig. 40, *A. lanata*, pistillate cymule, reconstruction of vascular system, adaxial tertiary bract present (*cf.* fig. 41). Figs. 41, 42, *A. crispa* var. *mollis*, pistillate cymule with adaxial tertiary bract present, floral diagram and habit respectively.

The series of schematic diagrams shown in figure 23 represent the possible transitions from the four-ovuled ovary to a potential one-ovuled ovary in the Betulaceae. The four-ovuled diagram may also be considered to represent the rarely observed five-ovuled condition. The fifth ovule is superposed on any one of those already present. Intermediate between the four-ovuled and the two-ovuled state is the presence of three ovules, a condition which was observed in a number of species. The three-ovuled condition could then give rise to either of the two-ovuled conditions shown, by the loss of one or the other of the ovules on the margins of the same carpel. No case has been observed of one of the carpels becoming completely sterile and the other bearing two ovules. Either of the two-ovuled conditions may occur within the same cymule (as shown in fig. 44, and 1, fig. 150). In most of the genera of the Betulaceae both of the ovules usually present develop somewhat beyond the fertilization stage, but frequently only one of these matures. This tendency reaches its extreme in *Corylus*, where two-ovuled fruits are rare. The trend in the family appears to be definitely toward the one-ovuled condition, representing the ultimate in a reduction series from a multi-ovulate pistil. Should a one-ovuled type of pistil ultimately become the rule in the family, the development of an equitant ovule (comparable with that of the Juglandaceae) is conceivable, although this is highly unlikely since it would necessitate the return of an anatropous ovule to an orthotropous condition.

4. Variations in number of stamens

Comment has already been made (1) on the variability in the number of stamens in the florets of *Alnus*, *Betula*, *Carpinus*, and *Ostrya*. The androecium in *Ostryopsis* and *Corylus* is usually variable within much narrower limits than in the other genera mentioned. The presence of an exceptional increase in the number of stamens in *Corylus* florets has already been described in discussing the presence of tepals in these florets. Schulz (**22**) reports the occurrence of unusual numbers of stamens in *Corylus avellana*, although he did not find tepals in his material. His observations may be interpreted as being examples of (a) reduction to monomery in each of the three

florets of the cymule, (b) monomery of the tertiary florets and trimery of the secondary floret, and (c) monomery of the tertiary florets and tetramery of the secondary floret. BAILLON (**8,** p. 225, footnote 3) mentions such variations in more general terms for *Corylus*, while WYDLER (**29**) early recognized the variability of stamen number in *Alnus*.

Monomerous androecia in the florets from the transition region of aments of *Alnus glutinosa* (**31**) and monomerous or dimerous androecia in *Ostrya carpinifolia* and *O. virginica* (**15**) have been noted.

In the remarkably reduced cymule of *Alnus* s. Cremastogyne there may sometimes be a total of five stamens instead of four distributed among the three florets (**21**), indicating either trimery of the secondary floret or dimery of one of the tertiary florets.

5. BISEXUAL FLORETS AND SEX REVERSAL

Bisexual florets in the Betulaceae are rare in the experience of the writer. One case, observed in *Betula luminifera*, is teratological since it is associated with insect injury. Here, in the same ament which produced the tricarpellary ovary already referred to, there also occurred a bisexual floret (fig. 33). A clearly recognizable stamen (*st*) is fused by its filament with the ovary wall. In this same floret the ovules (*ov*) are extruded through an enlargement of the interstylar canal, adding to the bizarre appearance of the floret.

Bisexual florets were also found in an apparently normal pistillate ament of *B. lenta*. The lowermost three or four cymules of one collection have individual florets which exhibit various conditions. The filaments of the stamens in some are long and fused, for most of their length, with the wall of the ovary with which they are associated—the anthers remaining free, however. In others the filaments are short, the anthers being practically sessile at the base of the ovary.

The third case in which bisexual flowers were noted was in the transition region of a pistillate ament of *B. pumila*. Here it is not clear whether the stamens represent the tertiary florets and the ovary a secondary floret, or whether all are associated together as members of the secondary floret. In several other specimens from the same region there appear to be staminodes opposite some of the

tepals. A similar condition occurred in *B. globispica*. The latter were the only cases where there were structures of any sort in the cycle between perigon and gynoecium, but the morphological identity of these is by no means clear.

In the many staminate cymules studied, a careful watch was maintained for external evidences of vestigial ovaries. These were not found, nor is there vascular tissue in the staminate florets which suggests a vestigial ovary supply. Other workers, however, have found hermaphroditic flowers in various species. BAIL (**4**) reports them in *Alnus incana* in the transition zone of a bisexual ament. In the staminate flower there was a rudimentary ovary between two of the stamens. In otherwise normal *A. glutinosa* he observed a similar condition, as well as in *Corylus avellana*. WORSDELL (**28**) found a single stamen in the female flower of *Carpinus betulus*. SCHULZ (**22**) observed a number of hermaphroditic florets. In the basal region of pistillate aments of *Alnus glutinosa* he found florets which, in their most complete state, had perigon and androecium similar to those of the staminate florets and a gynoecium like that of the pistillate florets (the orientation of the latter being either median or transverse to the primary bract). He also found hermaphroditic flowers at the base of staminate aments. In *Betula "alba,"* SCHULZ found florets at the base of staminate and pistillate aments, which, like those of *Alnus glutinosa*, combined the features of the usual staminate and pistillate florets. Interestingly enough, the orientation of the pistil here was also sometimes median to the primary bract.

In these florets the perigon and androecium were sometimes tetramerous. SCHULZ also found as a very rare occurrence in *Corylus avellana* the presence of hermaphroditic flowers at the base of a pistillate ament. Here the secondary floret of the cymule, which alone was present, had a tiny perigon, four stamens, and a bicarpellary ovary. VON MERCKLIN (**15**) figures and describes a bicarpellary ovary accompanied by two stamens inserted below the ovary in *Ostrya virginiana*. WEISS (**26**) found bisexual florets in the region between staminate and pistillate portions of androgynous aments of "hazel," as well as cymules in which the secondary floret is staminate and the tertiary ones are pistillate. Associated with anomalous branching of the staminate catkins of *Corylus avellana*, MÜLLER-STOLL (**16**) found

pistillate branches rarely developed, in one of which a pistil had two stamens accompanying it.

Two examples of sex reversal were observed by the writer, both in the transition region of a pistillate ament of *Betula lenta*. In the axils of two of the much reduced foliage leaves characteristic of this region there were stamens associated with tepals, forming a staminate floret instead of the pistillate floret which usually occurs in such positions (fig. 54).

WEHRLI (**25**) reports for *C. avellana* the complete replacement of the four stamens in otherwise normal staminate cymules, with an equal number (rarely three or five) of long red stigmas. This he considers evidence in support of the theory that the male and female elements of the flower (in general) are identical.

HÖSTERMANN (**13**) has found pistillate flower buds in the transition region of staminate aments in *Corylus*. He quotes PENZIG as reporting this phenomenon to be a regular occurrence in *C. maxima*. BAIL (**4**) found a staminate floret in the transition region of a pistillate ament of *Alnus glutinosa*.

ZIMMERMANN (**31**) has noted numerous cases of the replacement of anthers by carpels in staminate florets of *A. glutinosa*. He has also noted in the same species florets which have a tetramerous perigon, dimerous androecium, and dimerous gynoecium centrally located.

VON MERCKLIN (**15**) reported the replacement of one or of both anthers by one or two carpels in both *Ostrya carpinifolia* and *O. virginiana*. These occurred in the terminal region of a considerable number of staminate aments. The florets in this region were reduced to monomery, as is so often the case in the terminal portions of aments.

Inflorescence variations

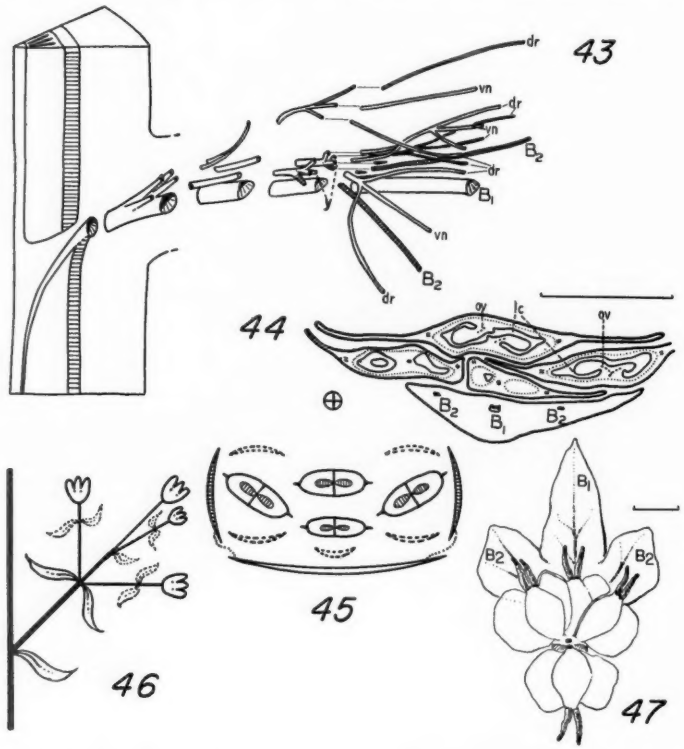
1. PRESENCE OF BRACTS USUALLY ABSENT

Bracts which are usually absent occur most frequently in the pistillate cymules of *Alnus*. Here one or the other of the adaxial tertiary bracts, more or less reduced, characterizes one or more collections of *A. jorullensis*, *A. crispa* var. *mollis* (fig. 42), and *A. lanta* (**1**, fig. 64). As many as half of the cymules in the ament may have an adaxial tertiary bract, especially in *A. crispa* var. *mollis*.

The most extreme development of the adaxial tertiary bract was found in *A. lanata* (1, fig. 64). Associated with its relatively large size is the clarity of the relationship of its vascular supply to that of the rest of the cymule. In figure 40 is shown that part of a three-dimensional reconstruction of the vascular system of the cymule mentioned which indicates the origin of the vascular supply to the adaxial tertiary bract (*adaxial* B_3). It should be noted that the bundle to the adaxial tertiary bract arises from a gap between the bundles *y* and *y*, which represent in part the vascular material of the tertiary axis. A strictly comparable situation characterizes the departure of the bundle to the adaxial tertiary bract in the pistillate cymules of *A. jorullensis*, when one or the other of these bracts is present. In *A. crispa* var. *mollis*, where the vestigial adaxial tertiary bracts are smaller, when present (fig. 42), the vascular system of the bract may be continuous with that of the rest of the cymule, or else may be entirely discontinuous if the adaxial tertiary bract is very small (1, figs. 25–34). In the various species which occasionally possess adaxial tertiary bracts, there seems to be a definite correlation between the absolute size of the vestigial bract and the extent to which it is vascularized, the structure still being recognizable after direct continuity of its vascular system with that of the rest of the cymule has been lost. This condition apparently represents an arrested state of the ordinary course of events in the development of the vascular system of a foliar organ. The further significance of the presence of the adaxial tertiary bract will be considered in connection with the presence of quaternary florets in *A. subcordata*.

There was found in the Arnold Arboretum a specimen of *Carpinus caroliniana* with an unusual development of small subsidiary pistillate aments in the axils of the variously reduced foliage leaves, immediately below the bases of the usual aments. A great variety of conditions was noted in the individual cymules. The subsidiary aments have from one to three or four nodes. The proximal cymule of the subsidiary aments is usually sterile and is represented by two large secondary bracts more or less fused with the small primary bract. In the second or third cymule there are from one to three florets, the most complete development of bracts and florets observed being shown in figure 50. In this cymule there is an additional

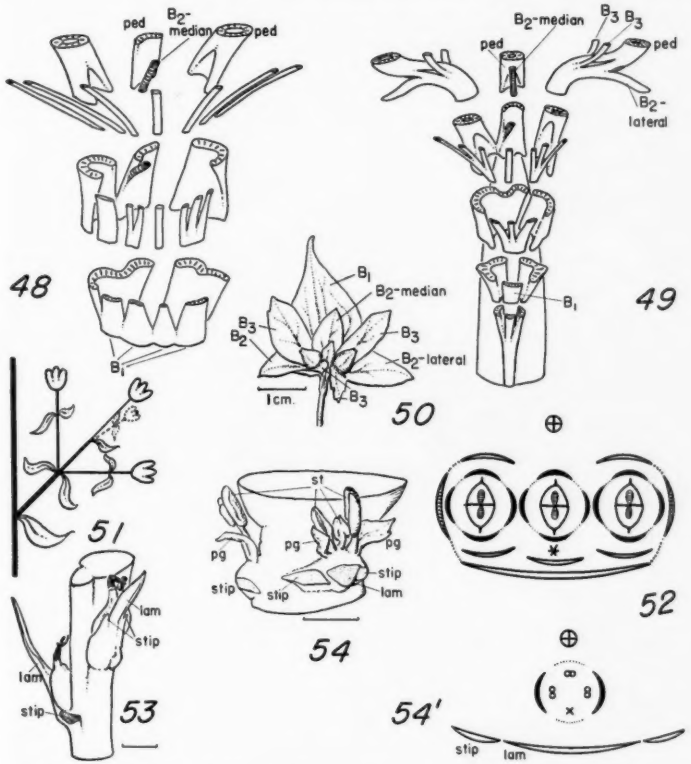
bract in the median plane which is designated as the median secondary bract (fig. 50, B_2-*median*). The vascularization of this particular cymule is shown in figure 48, where it will be noted that the trace



FIGS. 43–47.—*Betula papyrifera* var. *occidentalis*, four-flowered pistillate cymule; fig. 43, reconstruction of vascular system; fig. 44, cross section of cymule near middle of florets; fig. 45, floral diagram of cymule; fig. 46, diagram of inflorescence; fig. 47, habit sketch of cymule, uppermost floret turned down.

to the median secondary bract arises from the rather poorly defined vascular cylinder of the secondary axis. This suggests that the bract involved is at the second node on the secondary axis (fig. 51). Striking confirmatory evidence for this view comes from

the presence of a floret in *Betula*, which has a similar relationship to the vascular system of the secondary axis and will be discussed on a



FIGS. 48–54′.—Figs. 48–52, *Carpinus caroliniana*, pistillate cymule; fig. 48, reconstruction of vascular system of cymule shown in fig. 50; fig. 49, reconstruction combining characteristics of vascular systems of several extreme types of cymule; fig. 50, habit sketch of cymule whose anatomy is shown in fig. 48; fig. 51, inflorescence diagram of fig. 50; fig. 52, floral diagram of fig. 50. Fig. 53, *Betula schmidtii*, basal region of pistillate ament to show transition from reduced leaf with its stipules to their fusion. Figs. 54, 54′, *B. lenta*, staminate florets in axils of reduced foliage leaves at base of pistillate ament and floral diagram of anterior floret respectively.

subsequent page. ZIMMERMANN (**31**) describes in *Alnus glutinosa* a pistillate cymule which appears to have the median abaxial tertiary and quaternary bracts present, although this interpretation of his

figures and descriptions is open to question (see his numbers 19 and 20, 1918).

In staminate material of *Carpinus*, there has already been noted (1, figs. 239, 241 and discussion) the occurrence of free secondary bracts in the staminate cymules at the proximal end of the ament in *C. japonica*. ZIMMERMANN (31) noted this same occurrence in a species of *Carpinus* (*C. betulus?*). This evidence, in addition to the fact that the vascular system of the secondary bracts is present in the apparently simple "primary" bracts in most material of *Carpinus*, suggests that this primary bract is in reality a compound structure representing the lateral fusion of the primary bract with the secondary bracts.

The teratological staminate aments of *Corylus vilmorinii*, already referred to in the discussion of tepals, also have occasional cymules in which both the adaxial and abaxial tertiary bracts are well developed (figs. 31, 32). This is a significant case because the tertiary bracts are regularly absent in normal staminate material. In contrast, the tertiary bracts are fully developed in the pistillate cymules, forming the "involucral husk." All four of the tertiary bracts are not always present in the teratological staminate cymules, although at least two are generally represented.

The freedom of one or the other of the tertiary bracts from the secondary bracts is noted in pistillate *Carpinus betulus* var. *heterophylla* by ČELAKOVSKÝ (10), while he also notes variation toward the opposite extreme from the average; namely, nearly complete fusion of a tertiary with the secondary bract. PRANTL (19) observed the freedom of the adaxial tertiary bract in *C. japonica*, and indicates that this is the structure variously referred to by earlier taxonomists in their descriptions.

2. ABSENCE OF BRACTS USUALLY PRESENT

In the staminate and pistillate aments of *Alnus* and *Betula* and in the staminate aments of *Corylus*, the number of bracts usually present in the central portion of the ament is often reduced in the cymules at the extreme proximal and distal regions of the aments. This is illustrated by the absence of the secondary bracts in the single-flowered cymule from the distal portion of an ament of

Betula lutea shown in figure 9, as contrasted with the presence of the secondary bracts in a cymule from the central portion of the same ament (fig. 7). It is this type of phenomenon occurring normally in every ament under stress of space restrictions which makes more easily understandable the phylogenetic reduction from the type of cymule found in *B. alnoides* var. *pyrifolia* (**1**, figs. 97, 98) to the simpler type of *B. luminifera*. There is a similar transition from the pistillate cymules of the other birches of the Nanae group (**1**, figs. 80, 82) to those of *B. michauxii* (**1**, fig. 84), or from the staminate cymules of the alders where secondary and some tertiary bracts are present to those of *A. lanata* (**1**, figs. 177, 178), in which only the primary bract is evident externally. Intermediate stages occur in *A. firma* var. *hirtella* (**1**, figs. 156, 157) and in *A. maritima*, where the tertiary bracts are in a state of impending loss or else gone entirely as in some specimens of *A. crispa* var. *mollis* (**1**, fig. 158) and of *A. alnobetula* (**27**). This is given as a general condition in *Alnus*, subgen. *Alnaster*, series Firmae, by CALLIER (**9**) in his revision; his data for this characterization he credits to SCHNEIDER.

3. PRESENCE OF FLORETS USUALLY ABSENT

The occurrence of the secondary floret in pistillate cymules, where ordinarily only the two lateral tertiary florets are present, was found in *Alnus crispa*, *A. jorullensis*, *A. nitida*, *A. subcordata* (figs. 35, 36), *A. incana* (**22**), *A. glutinosa* (**22, 31**), and *Carpinus caroliniana* (fig. 50). The description and interpretation of the condition in *Alnus crispa* var. *elongata* (**20**) have been taken up in great detail elsewhere (**1**, pp. 6–9, figs. 8–19). The three-flowered cymules occurred on a plant, otherwise normal, whose pistillate aments are more or less elongate at the base. In this region occur a number of three-flowered cymules whose vascular anatomy is extremely advantageous for study and interpretation, since they are less telescoped than those of the normal ament. The evidence clearly establishes the fact that the central floret of the cymule is the secondary floret. It also provides a standard of comparison for the more reduced cymules of the central portion of the same aments as well as for the three-flowered cymules in other species.

Three-flowered cymules in the other species of *Alnus* mentioned

occurred in the central portion of the aments, where the secondary floret varies from nearly the usual size (*A. subcordata*, fig. 38) to a mere vestige (some of the specimens of *A. jorullensis* and *A. nitida*). In all of the three-flowered *Alnus* cymules which were sectioned, the vascular system was essentially similar to that described for *A. crispa* except for minor modifications induced by their dorsiventral compression. When the secondary floret is extremely reduced in size there is a corresponding reduction in the extent of its vascular supply. In some cases a vascular behavior simulating that of the pistillate cymules of *Betula* occurred. It is significant that WOLPERT (**27**) found rather frequently in early stages of the development of pistillate cymules in *Alnus* that a primordium representing the secondary floret is present, but later usually aborts, very rarely persisting to the mature stages.

The case of the presence of a secondary floret in *Carpinus caroliniana* (fig. 50) has already been described in some detail in connection with the presence of the median secondary bract. In other cymules than the one illustrated, a secondary floret is also often present. ČELAKOVSKÝ (**10**), discussing variation in the pistillate cymules of *Carpinus betulus* var. *heterophylla*, described several cases of the presence of the secondary floret in addition to the tertiary florets, as well as alone. In *C. betulus* three-flowered pistillate cymules were noted by SCHULZ (**22**).

A diagrammatic summary based on serial sections of three-flowered pistillate cymules of *C. caroliniana* studied by the writer is shown in the reconstruction (fig. 49). They are characterized in most cases by the development of an independent pedicellar cylinder (continuation of the secondary axis) which supplies the secondary floret. In one case, however, the secondary floret was supplied by only a single branch from the vascular cylinder of one of the tertiary florets, a well defined vascular cylinder not being formed. The vascular system of these three-flowered cymules of *Carpinus* is strikingly similar in its essentials to that of the three-flowered cymules of *Alnus crispa* (**1**, fig. 19).

Pistillate cymules of *Corylus* may also be three-flowered, owing to the presence of the secondary floret, according to SCHULZ's observations on *C. avellana* (**22**) and WEISS's observations on "hazel" (**26**).

The latter describes as an interesting variant a cymule in which the secondary floret was staminate and the tertiary ones pistillate.

An interesting case occurred in *Betula papyrifera* var. *occidentalis* where there are ordinarily three florets in each pistillate cymule. In several aments from one collection the greater number of cymules in the proximal portion of the ament have four florets (fig. 47; the uppermost of the two median florets is turned back to permit all four florets to be seen). The vascularization of this same cymule is shown in figure 43, from which it will be noted that the usual condition in this group (*cf.* 1, fig. 67) is augmented by a vascular supply to the second of the median florets. The bundles *y* and *y* continue on to vascularize the second median floret after contributing to the first median floret, and finally end in stubs, as do their homologues in *B. lenta* (1, fig. 67).

The vascular supply to the second of the median florets in the four-flowered cymules indicates clearly that this floret is attached to the continuation of the vascular system of the secondary axis of the cymule. This floret (fig. 46) is at a higher node on the abaxial side of the secondary axis in a position which corresponds to that occupied by the median secondary bract described in *Carpinus caroliniana* (fig. 51).

Another type of four-flowered cymule was encountered in the aments of *Alnus subcordata*, which have already been referred to as occasionally producing three-flowered cymules. The two additional florets lie side by side between the usual lateral secondary florets (fig. 34). In the reconstruction of the vascular system of this cymule (fig. 37) the vascular supply to the two unusual florets is indicated by stippling for the one and by cross-hatching for the other. The vascular system of the cymule departs from the plan characteristic for *Alnus* only in the presence of the bundles *f* and *f*, which develop into the pedicellar supplies of the two extra florets. Significantly, these pedicellar supplies originate from a gap left by the branching off of bundle *y* on either side away from *x* (which represents a fusion of the two central *y* and *y* bundles). A comparison of this mode of departure of the vascular supply to the two extra florets with the mode of departure of the bundle to the adaxial tertiary bract in *A. lanata* (*adaxial* B_3, fig. 40) suffices to demonstrate the close rela-

tionship between them. Here again in one species the foliar organ is present in the same location as that from which the supply to the floret arises in another species, and again it is possible to conclude that both originate from the same node. Thus there is present a quaternary set of florets in the axils of the adaxial tertiary bracts, where they might well be expected.

4. Absence of florets usually present

Mention has already been made of the consistent tendency in the subfamily Betuleae for the number of bracts and of florets per cymule to be reduced in the distal, and to a less extent, in the proximal regions of the ament. In the pistillate cymules of *Betula* the number of florets may be reduced from three to two (simulating pistillate *Alnus*), as noted by the writer in *B. nana* and *B. papyrifera* var. *occidentalis* and by Wolpert (**27**) for *Betula* sp. Or there may be a loss of the tertiary florets and the persistence of the secondary, the average condition in *B. michauxii*, occurring as a reductional extreme in the terminal portions of the aments in many species of *Betula*. The latter condition has also been noted by Schulz (**22**) in hermaphroditic cymules of *Betula "alba."* Similarly in *Alnus* the number of florets may be reduced to one in each cymule. Schulz notes this in certain pistillate cymules of *A. glutinosa* and *A. incana*. On the other hand, in the staminate cymules of *Alnus*, the secondary floret may be absent, as the writer has observed in *A. tenuifolia* (**1**, pp. 31, 32) and in *A. jorullensis* (*cf.* fig. 67), Schulz in hermaphroditic cymules of *A. glutinosa*, Zimmermann (**31**) in staminate cymules from bisexual aments of *A. glutinosa*, and Wolpert (**27**) in *A. alnobetula*.

In the Coryleae the writer has found reduction of the cymule to one floret only in *Carpinus caroliniana*, in the stunted aments at the bases of certain pistillate aments. An unusual development is described by Weiss (**26**) for "hazel," which had three-flowered pistillate cymules. Occasionally he found the three-flowered cymule reduced to the one-flowered state, the one flower representing the secondary floret, which in the average cymule is completely absent. Schulz (**22**) found staminate cymules in *Corylus avellana* in which only the tertiary florets (each monomerous) had developed, while

BAILLON (**8**) notes the occurrence in *Corylus* of only the secondary floret (also monomerous). In pistillate *Corylus* there is often not only a reduction of the individual cymule to one functional fruit (representing a tertiary floret) but also a functional reduction of the entire ament to this single fruit. While almost all of the cases of reduction observed result in a bilaterally symmetrical condition, the not infrequent behavior of pistillate *Corylus* stands apart because of the asymmetrical disposition of the fruit. This is, of course, associated with an earlier loss of the secondary floret of each cymule, followed by a further failure of one of the two tertiary florets to function.

5. FLORETS IN AXILS OF FOLIAGE LEAVES

Many examples of pistillate florets in the axils of reduced foliage leaves were found, notably in the transition region in *Betula*. The following were the source of one or more examples: *B. glandulosa*, *B. grossa*, *B. lenta*, *B. lutea*, *B. maximowicziana*, *B. michauxii*, *B. nana*, *B. nigra*, *B. papyrifera* var. *occidentalis*, *B. pumila*, *B. schmidtii*, and *Alnus hirsuta*. All of these were found in the transition region and showed in common the tendency for the presence of only a single floret in the axil of the reduced foliage leaf (figs. 3, 5, 25, 53), the floret in each case replacing the axillary bud. Only very rarely are secondary bracts present (fig. 3). The number of tepals associated with the floret varies from one to the next (figs. 3, 25), while the pistils are sometimes tricarpellate (figs. 3, 5, 53) or four-ovuled (fig. 24).

The leaves in whose axils these florets occur are generally restricted to the two or three nearest the ament proper and are much reduced, the leaf blade (*lam*, fig. 5) being of nearly the same size as the stipules (*stip*). Sometimes immediately below the ament the reduced stipules are fused laterally with the blade (*lam*, upper node, fig. 53), forming a single foliose organ equivalent to the primary bract.

In staminate material the transition region is but seldom productive of unusual cases, the one case observed in *Carpinus japonica* having been already figured (**1**, fig. 241). Here a single cymule composed of a small group of stamens occurs in the axil of a much

reduced foliage leaf. The only example of a solitary staminate floret was observed in the axil of a reduced foliage leaf in the case of sex reversal in *Betula lenta* (fig. 54), where a small group of stamens associated with tepals occurred at two different nearby nodes.

A rare occurrence is that described by GERTZ (11) for *Alnus glutinosa*. He found in the lower part of an elongated pistillate catkin the individual florets replaced by miniature pistillate aments, some of which even had a "transition region." This is a noteworthy occurrence, indicating that even in the much reduced *Alnus* pistillate flower there still exists the potentiality for development into a more extensive leafy axis (in this case fertile).

ZIMMERMANN (31) describes in *Alnus glutinosa* numerous cases of solitary florets which are staminate, pistillate, and hermaphroditic in the axils of reduced foliage leaves in the transition region. He also observed solitary staminate and pistillate florets in a similar position in *Betula*.

6. MIXED INFLORESCENCES

A number of partly pistillate and partly staminate aments have been reported. They are especially interesting in the transition zone from staminate to pistillate cymules, since hermaphroditic florets sometimes occur there (noted previously under the heading Bisexual florets and sex reversal). HÖSTERMANN (13) noted a pistillate inflorescence of *Corylus maxima* which was staminate in the upper portion. BAIL found similar situations in *Alnus incana* (4), *Betula "alba"* and *B. humilis* (3); SCHULZ (22) reports bisexual aments in *Alnus incana* and *A. glutinosa;* BAILEY (5) for *Alnus "serrulata";* and BAILLON (7) for *Alnus* sp.

Specimens of *Alnus glutinosa* in which several pistillate aments became staminate in their upper portions and one case of the reverse condition have been noted by ZIMMERMANN (30, 31). In the transition from one sex to the other he found numerous bisexual flowers and other aberrations, many of which have been mentioned here under the appropriate headings.

Pistillate inflorescences occurred in the center of groups of bunched staminate aments in *Corylus avellana*, according to MÜLLER-STOLL (16).

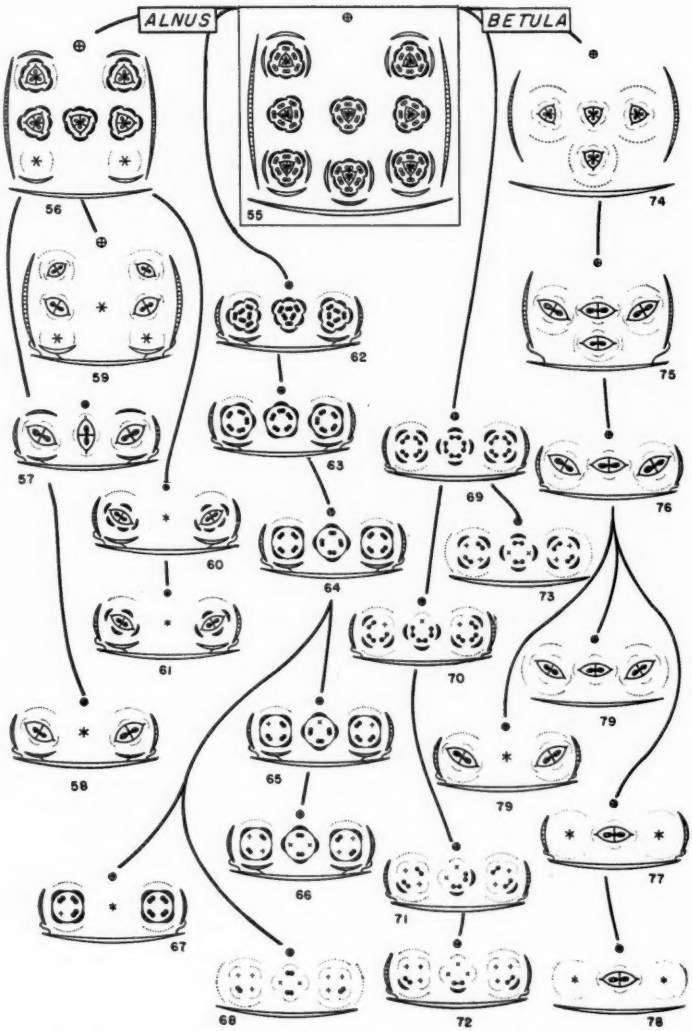
Range of floral variation in the Betulaceae

It is now possible to summarize the range of variation in the florets and inflorescences of the Betulaceae. This is shown diagrammatically in figures 55–100, with reference throughout to a synthetic floral diagram (figs. 55, 80) which is a summary of the average conditions plus the more complex variations. Dotted lines are used for the organs which have not been found, but which serve to complete the cymose plan. In each genus the range of variation is shown as progressing from the more complex (presumably the more primitive) conditions through various stages of simplification (reduction). It is not implied that the species which form the basis for any of the diagrams are progenitors of other species represented by other diagrams. The species cited merely form the basis for an estimate of the morphological changes taking place in the family. A phylogenetic classification will be suggested at a later date, when the evidence from fields other than floral structure has been presented. Morphologically the family is of great interest because of the wide range of variation, the florets sometimes varying from hexamery to monomery and from syntepaly to atepaly within the same genus, and the cymules ranging from a four-flowered to a one-flowered state with corresponding reduction in number of bracts.

It is to be understood throughout this discussion that statements concerning conditions which are described as characteristic of certain subdivisions of the family are based on the observations of the writer. While the observations of others have been referred to and an attempt made to interpret them in the main body of this paper, the writer does not include them in this section, since the data provided are often insufficient to serve as a basis for complete floral diagrams.

Betuleae (figs. 55–79)

Alnus, pistillate cymules (figs. 56–61).—The more complex pistillate conditions are summarized in figure 56. Here is embodied the tricarpellary condition of the pistils from the transition region, the presence of a perigon, and the presence of adaxial tertiary bracts and quaternary florets, all of which are extremes found in one or another of the species of the genus. The perigon is shown as hex-



FIGS. 55–79′.—Diagrammatic summary of range of variation in florets and cymules of Betuleae.

amerous because the conditions observed in *A. rubra* may well be derived from such an arrangement, and it is found on occasion in the staminate florets of other species. Syntepaly is indicated because the presence of glands on the upper part of the ovaries suggests an even more complete fusion of the perigon with the pistil than that which is associated with syntepaly in the Coryleae. The average type of two-flowered pistillate cymule is shown in figure 58. This could be derived morphologically either from the four-flowered condition shown in figure 59 (based on *A. subcordata*) by the loss of the quaternary florets, or from figure 57 (based on *A. crispa* var. *elongata* and *A. subcordata*) by the loss of the adaxial tertiary bracts and of the secondary floret, or from figure 61 (based on *A. rubra*) by the loss of the tepals. While there is a considerable range of variation in the pistillate cymules of *Alnus*, the great majority of average cymules for each species studied falls in with the diagram shown in figure 58.

ALNUS, STAMINATE CYMULES (FIGS. 62–68).—There is great variation in the average condition of the individual florets and practically no variation in the number of florets per cymule from one section to the next. Thus, in the s. Alnobetula, florets tend to be hexamerous or pentamerous (figs. 62, 63), with occasional further reduction to tetramery or trimery, while one or both of the abaxial tertiary bracts may frequently be absent (not shown in the diagrams). In the s. Gymnothyrsus, tetramery (fig. 64) or trimery (fig. 65) is frequent, with occasional dimery (fig. 66), all with the abaxial tertiary bracts usually present. In the s. Clethropsis the individual florets range from pentamery (fig. 63) through trimery (fig. 65), the cymules usually having the abaxial tertiary bracts present. In a phylogenetic scheme the members of this section would follow a separate line because of the marked abbreviation of the cymules in length, a characteristic which does not lend itself to representation in the floral diagrams. Finally, in the interesting s. Cremastogyne (fig. 68), not only are the tertiary bracts absent as they so often are in the s. Alnobetula, but the androecium is monomerous or dimerous as in the more reduced members of s. Gymnothyrsus, the cymule is short like that of the species of s. Clethropsis, and, unlike any of the other members of the genus, the secondary bracts are absent and the

florets are atepalous. In each of these groups, except the last, the average conditions are flanked by extremes which parallel in one or more ways the average condition in the other groups. One type of variant (fig. 67, based on *A. tenuifolia* and *A. jorullensis*) which occasionally develops is of interest because of the absence of the central floret, thus resulting in a situation like that of the pistillate cymule.

BETULA, PISTILLATE CYMULES (FIGS. 74–79′).—Figure 74 represents a synthesis of the more complex variations observed in this genus and embodies, in addition to the average structure, the presence of the abaxial median tertiary floret and of trimerous gynoecia. The four-flowered cymule (fig. 75, based on *B. papyrifera* var. *occidentalis*) is of peculiar interest, since it expands the median portion of the synthetic diagram for the family (fig. 55) into a several-flowered group, thus supplementing the contribution from the four-flowered cymules in *Alnus* (fig. 59). By the loss of the abaxial median tertiary floret the average condition in the ss. Costatae, ss. Albae, and part of the ss. Nanae is reached. By the further loss of the lateral tertiary florets (fig. 77, based on *B. nana*), followed by the loss of the secondary bracts (fig. 78, based on *B. michauzii*), the extremes in range of average conditions in the ss. Nanae are realized. In the ss. Acuminatae the average condition varies from that of figure 76 (as in *B. luminifera*) to the incipient and finally complete absence of the secondary bracts (fig. 79′, based on *B. alnoides* var. *pyrifolia*). A variant, characterized by the absence of the secondary floret, is of interest because it simulates the average condition in *Alnus* pistillate cymules (fig. 79, based on *B. nana* and *B. papyrifera* var. *occidentalis*).

BETULA, STAMINATE CYMULES (FIGS. 69–73).—There is a continuous series from the apotepalous tetramerous to a monomerous condition in both perigon and the androecium. The tetramerous state (fig. 69, based on hybrid [?] *B. maximowicziana*) is unusual and gives way to the trimerous (fig. 70) and dimerous (fig. 71) florets characteristic of the members of the ss. Costatae (with trimery not infrequent) and the ss. Albae (with dimery of the androecium and monomery of the perigon more frequent than in the ss. Costatae). In the ss. Nanae dimery of androecium and monomery of perigon

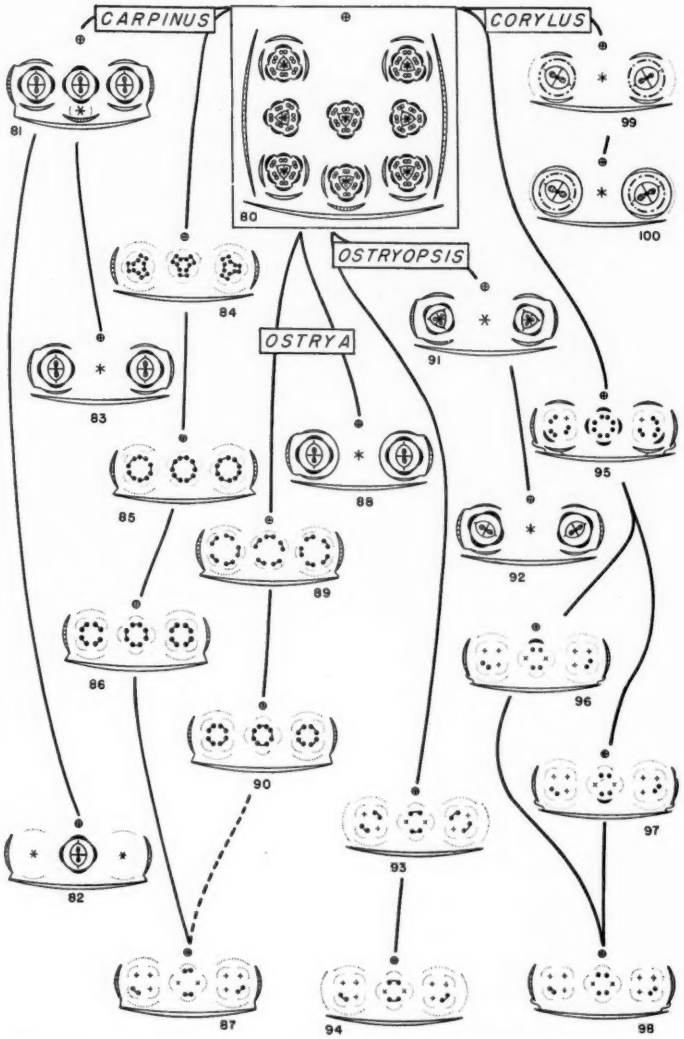
finally go over into monomery of both (fig. 72, based on *B. pumila* etc.). Divergent (except for *B. maximowicziana*) from these sections is the ss. Acuminatae (fig. 73) in the loss of the secondary bracts (present in all of the other ss.) and the tendency of the androecium to be reduced in number of parts before the perigon suffers such loss. In this latter respect the ss. Acuminatae shows an interesting parallelism to the occurrences in the staminate florets of *Alnus*.

Coryleae (figs. 80–100)

Carpinus, pistillate cymules (figs. 81–83).—The average condition in both sections of the genus is that shown in figure 83. Somewhat less reduced is the situation in which the secondary floret is present (fig. 81, based on *C. caroliniana*) and rarely accompanied by the abaxial median secondary bract. The latter circumstance is of especial interest in reconstructing a hypothetical ancestral form (figs. 80, 55), since it is in the axil of a bract in this location that the fourth floret occurred in one extreme case in pistillate *Betula* (fig. 75). A rare extreme in reduction is due to the loss of the tertiary florets and of the tertiary bracts (fig. 82, based on *C. caroliniana*). The latter is of interest not only because it represents a radically different organization from that of the average cymules in the genus (fig. 83), but also because it parallels an extreme of reduction found in the pistillate cymules of *Betula* (fig. 77).

Carpinus, staminate cymules (figs. 84–87).—The individual florets range from a hexamerous (fig. 84) to a dimerous or monomerous (fig. 87) state, depending on their location in the ament, and are notable in contrast with most of the Betuleae because of the absence of the perigon. It is, however, of interest to find that the same extreme of reduction is reached here as in some species of the Betuleae, although it is not so fixed that it becomes a species character. While the secondary bracts are mostly so completely fused laterally with the primary bract as to be indistinguishable externally, occasionally they are free, as indicated in figure 84 (based on *C. japonica*).

Ostrya, pistillate cymules (fig. 88).—Very little variation was observed, the florets and cymules consistently assuming the condition shown in figure 88.



FIGS. 80–100.—Diagrammatic summary of range of variation in florets and cymules of Coryleae.

OSTRYA, STAMINATE CYMULES (FIGS. 89, 90).—The range of variation closely parallels that in the staminate cymules of *Carpinus*, with the exception that the largest number of parts per floret is apparently less than it is in *Carpinus*. No examples of free secondary bracts have come to the writer's attention, although there is internal vascular evidence of their existence.

OSTRYOPSIS, PISTILLATE CYMULES (FIGS. 91, 92).—Occasional three-carpelled ovaries occur (fig. 91, based on *O. nobilis*), providing a transition to the usual two-carpelled ovary (fig. 92).

OSTRYOPSIS, STAMINATE CYMULES (FIGS. 93, 94).—The extent of reduction of the staminate florets is remarkable because of the strong resemblance between the pistillate florets of the genus and those of *Carpinus* and *Ostrya*. Yet there is in the average staminate florets of the genus a state of reduction to dimery (fig. 93) or monomery (fig. 94) which corresponds in extent only to extreme conditions in *Carpinus* and *Ostrya*. In this respect the floral structures correspond more closely to those of *Corylus*, although *Corylus* still retains the secondary bracts which are not evident (even internally) in *Ostryopsis* (*cf.* also fig. 68).

CORYLUS, PISTILLATE CYMULES (FIGS. 99, 100).—In both groups of species, those with the bracts fused laterally to form the "husk" (fig. 100) and those in which the bracts are free (fig. 99), the structure is uniform. The extraordinary absence of secondary bracts while both of the tertiary bracts are still present is also a very constant feature. The variation in the basic morphology of the florets is slight.

CORYLUS, STAMINATE CYMULES (FIGS. 95–98).—The average condition is that shown in figure 98, a combination of monomery and dimery in the various florets. In some cases perigon segments occurred (figs. 96, 97) which form a transition to the usual completely atepalous florets. The least reduced state (fig. 95, based on *C. vilmorinii*) is not only supplied with perigon in the florets but the usually absent tertiary bracts are also present in their entirety.

Discussion and conclusions

Although a consideration of the phylogenetic ramifications within the family is best left until other lines of evidence have been brought

to bear upon it, it is still desirable to attempt the synthesis of an ancestral type of floret and cymule. The present effort will be restricted to the reconstruction of a hypothetical form which combines the least modified characteristics observed in the various members of the family.

The question arises as to the validity of including in such a reconstruction the deviations from the average which are described in the main body of this paper. The objection which will be raised most frequently is that these deviations are *abnormal*. Normality is a difficult state to define objectively. Moreover, it is easily confused with the more restricted concept which may be referred to as *average*. In the previous paper of this series (1) it was the average condition which was stressed; namely, the condition which exists in most of the cymules in the major portion of the ament. But in these same aments, mostly at the base or apex, there occur cymules whose structure is either more simple or rarely more complex than that of the rest of the ament. These less usual forms must be considered as normal, since they uniformly occur in association with average cymules. They are slightly different expressions of gene complexes which presumably are the same in every vegetative cell of the plant. The difference in expression of similar gene complexes can best be attributed to slightly different spatial relationships of the parts involved, and perhaps to slight physiological differences. Thus the identical gene complexes in two adjacent florets of the cymule may be associated in one floret with the presence of only two tepals while in its neighbor there may be three tepals. Or, in another case, a floret in the apical region of the ament may have but one tepal; a floret in the central region, three; and a floret at the base, four tepals—apparently correlated with difference in position and probably also with physiological factors. Yet in all of them the genic complement is almost certainly alike throughout. All of these states are normal, but that which occurs most frequently, and thus is characteristic of the species, is to be regarded as the average.

Of the two extremes of variation from the average in a species, that of reduction is most easily recognized as foreshadowing the average in closely related species. In blocking out phylogenetic trends, at least these reductional extremes may be considered sig-

nificant. Good circumstantial evidence supporting this assumption is to be found in the pistillate cymules of *Betula*. Composing the average pistillate cymule of *Betula* ss. Albae, there are three bracts and three florets (fig. 76); yet in the same ament in which such cymules predominate, there are more reduced cymules in the crowded apical region of the ament. These latter cymules may sometimes be two-flowered (fig. 79) or in other cases one-flowered (fig. 77). In the ss. Nanae, with its smaller aments, this same range of variation is also frequently found. Often the entire ament will be composed of uniflorous cymules (as for instance in *B. nana*), but with each floret there is ordinarily the usual set of three bracts. In the terminal region of these aments, however, there may be only a single bract associated with the single floret (fig. 78). This latter condition, extreme in *B. nana*, is the average for *B. michauxii*. Briefly, the reductional extreme occurring in a region of crowding in one species is the average condition in the next more reduced species.

Not only are the extremes of reduction significant, but the opposite extreme often reflects the average in a less reduced species. But in the least reduced species this involves the presence of one or more organs which are absent in the average state of that least reduced species. Specifically, into this category in the Betulaceae come the florets which occur in the axils of the adaxial lateral tertiary bracts, the abaxial median secondary bract and the floret in its axil, the third carpel in the pistil, and the bisexual type of floret sometimes provided with a perigon. When these structures occur, they are in practically all cases associated with average conditions to the same extent as the opposite extreme (reduction). There is no more reason to assume that there are different genic factors involved in the development of these more complex cymules than in the development of more reduced ones. Furthermore, the less reduced conditions fit in at one end of the average range of variation as smoothly as do the more reduced states on the opposite end (figs. 55–58, 55–78, etc.). These more complex cymules may represent simply the result of interaction between more favorable external influences and the same internal hereditary factors that are expressed to a less degree in the reduced cymules of the same species under less favorable circumstances. It would seem that the less reduced conditions

are as significant in their phylogenetic implications in the Betulaceae as are the more reduced conditions.

The use to which these extremes of non-reduction may be put is clear. From each of the genera come fragments of evidence pointing toward a less modified type of floret and cymule. By fitting the bits of evidence together, a hypothetical ancestral type may be reconstructed. As shown in figures 55 and 80, this is a less reduced cymule than the average for the family. It should be noted that the hexamerous state of the individual florets is considered by the writer to be merely a stage in a reduction series from some remote, less reduced ancestral form which had a larger number of tepals in the perigon. The trimerous state shown represents the arrangement naturally taken by the tepals when only six are present and distributed on the basis of well known phyllotactic laws. There is no implication of monocotyledonous affinities in the trimerous state indicated; it merely represents the uppermost point in the wide range of variation characteristic of the family.

The different genera contribute various items to the reconstruction of a hypothetical ancestral form. From pistillate *Alnus* there are added to the basic three-flowered cyme the two adaxial quaternary florets and the adaxial tertiary bracts; from pistillate *Betula*, the abaxial median tertiary floret; from pistillate *Carpinus*, the abaxial median secondary bract. The remaining florets are added because the bracts subtending them are present, and because it is assumed that the cymule is, as its name suggests, basically cymose. That the latter may be a gratuitous assumption will be taken up in more detail elsewhere.

The individual floret of the reconstruction derives its two-cycled, trimerous perigon and androecium, as well as its syntepaly, primarily from staminate *Alnus*. The insertion of the stamens is in doubt, but was probably at the base of the free part of the tepal. The validity of indicating the floret as hermaphroditic is suggested by the cases of this sort observed in the transition from staminate to pistillate portions of androgynous aments, etc. The tricarpellary pistil is based on such pistils in *Alnus*, *Betula*, and *Ostryopsis*. The inferior tricarpellary ovary is based on *Ostryopsis*. The placentation is indicated as axile because this is the condition in the well developed

tricarpellary pistils which have been observed. The ovary probably had three completely distinct loculi. The number of ovules in each carpel was at least three (*cf.* figs. 1 and 2).

In attempting to allocate a position to the Betulaceae among the groups to which it is allied, these groups might be expected to have inflorescences and florets which can be derived from a plan closely similar to that worked out here for the Betulaceae. The Fagaceae come to mind, but further consideration of this problem must await a broader basis for comparison.

Summary

1. A number of the cymules and florets whose complexity is greater or less than the average in the Betulaceae are described. The more complex cymules include the presence of: the secondary median floret in pistillate *Alnus* and *Carpinus;* adaxial quaternary florets in pistillate *Alnus* and adaxial tertiary bracts in pistillate *Alnus* and staminate *Corylus;* the abaxial median secondary floret in pistillate *Betula;* and the abaxial median secondary bract in pistillate *Carpinus*. The more complex florets include the presence of: tricarpellary pistils in *Alnus*, *Betula*, and *Ostryopsis;* a hexamerous perigon and androecium in staminate *Alnus;* and three ovules per carpel in *Alnus*. A few cases of hermaphroditism were observed.

2. The more reduced conditions in the cymule include the loss of: the secondary floret in staminate *Alnus* and pistillate *Betula*, the tertiary florets in pistillate *Betula* and pistillate *Carpinus*. The more reduced conditions in the floret include the reduction of the androecium to monomery and the perigon to atepaly.

3. The less reduced conditions are summarized in a synthetic ancestral form. The characteristics of the cymule of this ancestral form would be as follows: a series of racemosely arranged cymose (?) inflorescences, each composed of a median triflorous group and two lateral triflorous groups, with an appropriate complement of bracts; the individual hermaphroditic flowers equipped with hexamerous perigon (probably syntepalous), hexamerous androecium, the gynoecium inferior, tricarpellary, with axile placentation and three or more anatropous ovules.

It is a pleasure to acknowledge the help derived from discussing this problem with Professor F. K. BUTTERS of the University of Minnesota, Professor A. J. EAMES of Cornell University, and Professor R. H. WETMORE of Harvard University.

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A PHOTOKYMOGRAPH FOR THE ANALYSIS OF THE AVENA TEST

C. L. SCHNEIDER AND F. W. WENT

(WITH FOURTEEN FIGURES)

I. Introduction

In the *Avena* technique, when an agar block containing auxin is placed unilaterally on the cut surface of a decapitated coleoptile, it will bend because of the increased growth of the side under the agar block. This technique, the *Avena* test, is extensively used for the quantitative determination of auxin. This paper describes results obtained with a photokymograph which was designed to record the procedure of these curvatures automatically.

It is known that, under prescribed conditions, these curvatures are proportional to the concentration of auxin in the blocks. This proportionality has been described by WENT (15), VAN DER WEIJ (13), VAN OVERBEEK (8), and SKOOG (11). Nonconformity, however, was found by NIELSEN (7) and SÖDING (12). Since the work of VAN DER WEIJ, no systematic analysis of the conditions most favorable for the test has been carried out, and the accepted procedure is partly a matter of chance. Thus a more detailed analysis of these curvatures has a practical value, besides its theoretical significance.

The first attempts to express the auxin curvature as a function of time were made by DU BUY and NUERNBERGK (1) and by DOLK (2[1]), in both cases by measuring the pictures of curving plants on film strips taken by a lapse time movie camera. The measurement and calculation require an excessive amount of work and time, however, so that this method has fallen into disuse.

Both VAN OVERBEEK (10) and SKOOG (11) give data on the procedure of the auxin curvatures under different conditions; but the time intervals between successive observations are long (one hour or more) and the measurement (with a protractor) does not allow for high precision, as required if finer details of the curvatures have to

[1] See figure 51 in WENT and THIMANN (17).

be analyzed. Besides, VAN OVERBEEK (10) has shown that the light necessary for photographing the plants influences the subsequent stages of curvature.

II. Method

The most desirable method of recording the procedure of the auxin curvatures ought to give a continuous permanent recording without

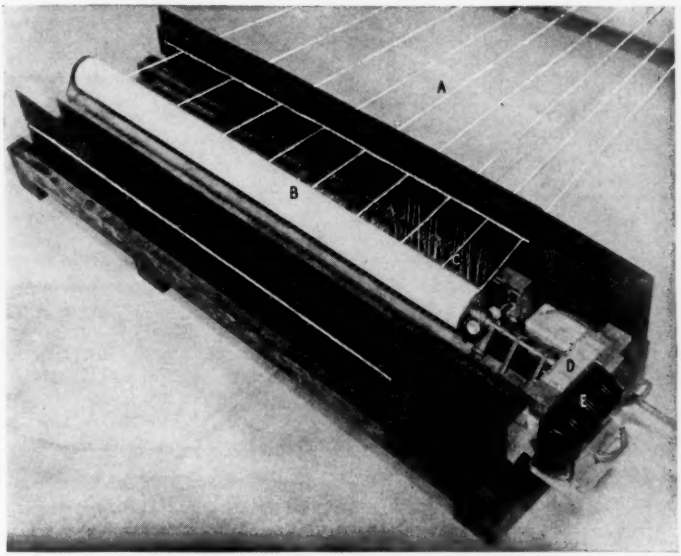

FIG. 1.—Photokymograph for automatically recording auxin curvatures in *Avena*. An intermittent beam of light, *A*, passes through the horizontal slit, striking the photographic paper on the rotating drum, *B*. Projections of the coleoptiles, *C* (straws inserted in place of primary leaf) interrupt the beam, leaving a photographic record of their positions. Speed of rotation of drum *B*, which is driven by hour-hand of electric clock, *E*, can be reduced to any desired period by gear box, *D*.

influencing the plants by exposure to light. Such a method is to be found in the use of the photokymograph (fig. 1). In the most satisfactory type yet used, this apparatus consists of a drum covered with photographic paper and rotating on a horizontal axis. A 1 mm. wide beam of light strikes the total length of the drum at the level of its axis at three or six minute intervals. This beam is obtained by

passing the light of a straight vertical filament of an automobile lamp through three successive horizontal slits, the last one being at a distance of 5 cm. from the drum. Thus practically no stray light comes into the room, especially not into the space between the last screen and the drum, where the plants are placed.

Plants of *Avena* are grown in the usual glass holders, which are assembled in rows of twenty-two plants each, a number of rows being placed along each side of the drum. The machine shown in figure 1 is large enough to accommodate 176 plants. The agar blocks with auxin are put on one side of the cut surface of the decapitated coleoptiles so that the coleoptiles will curve in a vertical plane parallel to the axis of the drum. A record of the angular movement of the curving coleoptile is obtained by photographing the displacement of the straw which is inserted in the place of the primary leaf. This straw, a 6 cm. peduncle of Bermuda grass (*Cynodon dactylon*), makes it possible to avoid exposing the plants to white light in taking the photographs, since only a 1 mm. strip 5 cm. above the curving zone of the coleoptile is illuminated. Projecting the moving tip upward 5 cm. by means of this peduncle also gives an enlargement of the displacement through its lever action. This peduncle with its surrounding leaf sheath just fits the coleoptile, can be adjusted in length by pulling it part way out of its leaf sheath, serves as support for the agar block, and does not bend hygroscopically. An alternative method is to force a fine ni-chrome wire into the partially pulled out and decapitated primary leaf. If the light source is a single filament, as in the lamps here described, then a sharp recording is obtained. This wire has the same advantages of lightness and rigidity as the peduncle just described, and is easier to fit into place. Whether the peduncle or the wire is used, it is inserted about 1.5 mm. into the coleoptile, thus attaching it firmly and yet not allowing it to interfere with the curvature by having a rigid body in the coleoptile.

In this way the position of each plant is indicated on the photographic record as an interruption in the black line caused by the light beam (fig. 2). The curving of the plant is shown by a shift of this interruption in successive recordings. The displacement of the projected coleoptile from its original position can be measured quite accurately (a standard shadow mark of a wire in the rack is used as

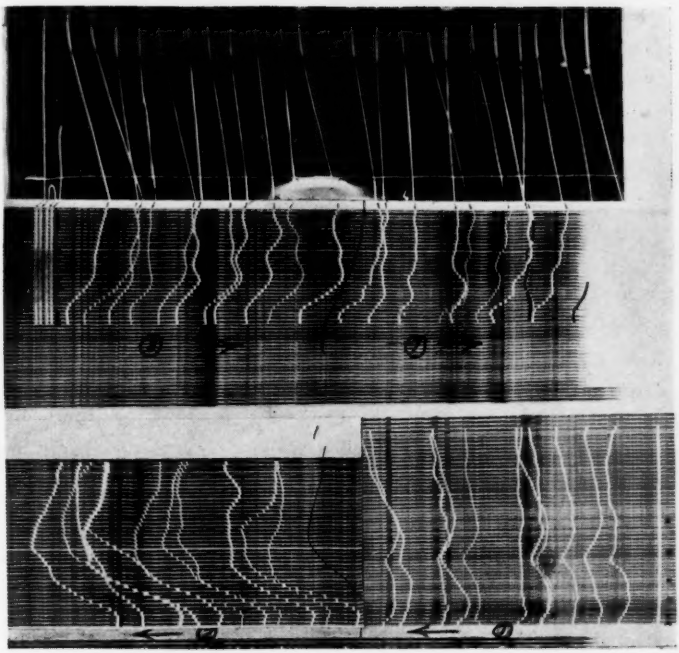

FIG. 2.—A record from the kymograph, with a recording every six minutes. Curvature of coleoptiles shown by displacement of interruptions in black lines in successive recordings. Broad vertical markings at ends of rows are reference marks left by the wire in plant rack. Four treatments shown in this record: in treatment no. 7 a plant has been marked out because of failure of contact between the block and cut surface. Nutations, seen especially in nos. 7 and 8, averaged out in measuring because various wave peaks are out of phase with one another. Arrows indicate direction of negative curvature, which is opposite on opposite sides of drum. Treatments 1, 7, and 8 had low concentration auxin applied, 0.03 mg./l. Treatment 1 had a very short (8 min.) i.d.p. and shows positive curvature; 7 and 8 had a long i.d.p. (220 min.), and show practically no positive curvature. No. 8 had double decapitation and gives more uniform results than single decapitation method of 7; it does not always give so great an increase in sensitivity as in this case. Treatment no. 2 is from another experiment and shows strong curvatures obtained with higher concentrations.

base line), and changes with the tangent of the angle of curvature of the plant. For small angles, 1 mm. corresponds to about 1° of curvature, and as fractions of a mm. can be measured, this method makes it possible to determine small curvatures which cannot be measured in the usual way. At the end of each experiment (usually three to six hours in duration) a shadow picture of the experimental plants is taken to determine whether the contact between agar and plant was good and whether the curvatures were normal.

As will be seen from the photokymograph record (fig. 2), the plants show nutations. These vary in amplitude from test to test, and the various wave peaks for the individual plants occur at different times so that they can be averaged out of the measurements by having several plants in each test. The nutations become less marked as the growth rate decreases, and seem to be the result of a delicately balanced system that is always righting itself and then overshooting the mark so that it becomes necessary to right itself again and again, as in the description of the autotropism of DOLK (**2**). A similar view was held by GRADMANN (**3**), who considered nutations as "Überkrümmungen."

As has been determined before (**13, 15, 5**), there is considerable variation in the sensitivity of *Avena* seedlings to auxin. The variation is of two kinds. First is the variation with age. This could be eliminated from the experiments by always choosing plants of the same age (3 cm. long and grown under the same conditions of light, temperature, and humidity).

Second is the variation with some as yet unknown external conditions. These can be only partly eliminated from the experiments, for they too are of different kinds: hourly variations (24 hour cycle), daily variations (apparently not cyclic), and seasonal variations (yearly cycle). The hourly variations may be eliminated by always having the test plants ready at the same time of day. Not much can be done to avoid the daily variations since they are as yet unpredictable. The annual variations can be eliminated by running the series of experiments within a period of a few days; or, as was actually done in the work for this paper, by repeating the experiments during different times of the year. This was easy to do since the responses happened all to be of the same general form, although of different

magnitudes. The effects of the unpredictable daily variations could also be eliminated by comparing the experiments of several days, since they too were variations in magnitude of response.

The results described in this paper were all obtained under the following conditions: temperature 24° C., humidity 85–90 per cent, and illumination by only occasional red or orange light (from incandescent lamps filtered through a Corning filter 348). The *Avena* seeds (Victory oats), obtained from Sveriges Utsädesförening, Svalöf, Sweden, were dehusked, soaked for one hour in water, and germinated for 30 hours on wet filter paper in dim red light to suppress subsequent mesocotyl growth. Then they were planted in the glass holders and grown in a dark cupboard for 45 hours. About 75 hours after soaking, the coleoptiles were 25–30 mm. long, at which length they were generally used. In the tests a 5–6 mm. tip was removed (unless otherwise stated).

The growth hormone used throughout these experiments was heteroauxin, indole(3)acetic acid obtained from Merck & Co. It was found that a standard solution could be kept for many months by the simple expedient of sterilizing it and keeping it in darkness. Since concentrated solutions lost activity rapidly if kept at high temperatures, the sterilizing process was modified by heating a measured volume of water for the solution (a five gallon bottle with water was heated almost to boiling in a waterbath for several hours), then allowing it to cool to about 70°, at which temperature the auxin crystals were added and the resulting solution mixed and cooled rapidly to room temperature. This sort of sterilizing produced a fully active solution which retained its activity. A siphon and burette previously sterilized with alcohol were then connected, and at any time desired, volumes of an auxin solution of known activity were at hand.[2]

Agar blocks were prepared by cutting 1 mm. slices with a microtome and preserving them in 50 per cent alcohol. Before using, they were washed in running water for one hour and then soaked in the auxin solution to be tested for another hour. They were then ready to be cut into the 7 mm.[3] sized blocks used in the experiments.

[2] While this paper was in press, some stocks prepared in a similar manner lost part of their activity, indicating that further conditions not yet known have also to be controlled.

III. Results

A. Avena test for low auxin concentrations

To test the effect of the time interval between decapitation of the *Avena* seedling and putting on of the auxin-agar block,[3] the following tests were made.

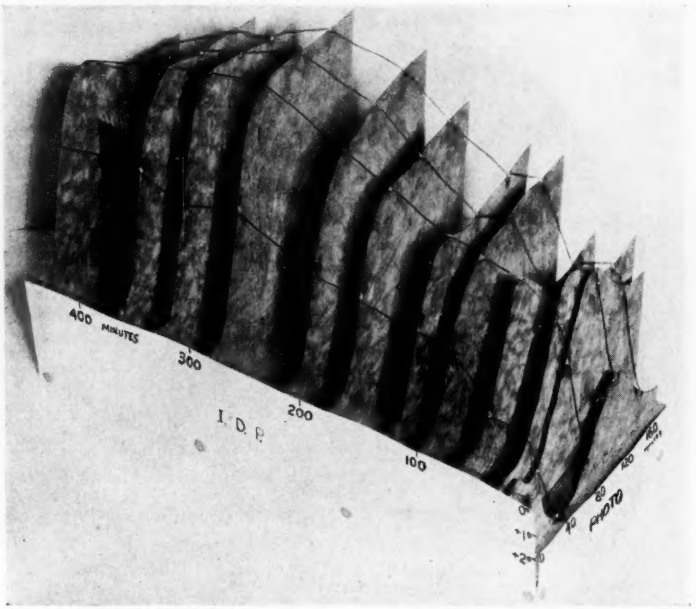

Fig. 3.—Three dimensional model for i.d.p., time after putting block on, and curvature, when a low auxin concentration (0.03 mg./l.) is applied unilaterally. The model is an average of four experiments. Each curve represents the reaction of approximately 30 plants.

At various times after decapitation a low heteroauxin concentration (0.03 mg./liter) was unilaterally applied to decapitated seedlings. The progress of curvature for each i.d.p. was drawn as a graph, and the resulting series of graphs was united into a three dimensional model (fig. 3). The ordinates represent the degree of cur-

[3] The *i*nterval between *d*ecapitation and *p*utting on is abbreviated as i.d.p. throughout this paper.

vature; the abscissa (to the right) the time in minutes after putting on the agar block; the third axis (toward the left) the time between decapitation and putting on of agar block (i.d.p.). This diagram was made as the average of the four experiments of August 14, 17, 20, 24, 1936; all giving the same form of curves and giving the same absolute curvatures. The diagram applies only to low concentrations, 0.03 mg./l.; for higher concentrations another set of results was obtained and will be described later. This low concentration model shows the following:

1. If blocks are put on soon after a single decapitation, a positive curvature is induced which is later replaced by the negative curva-

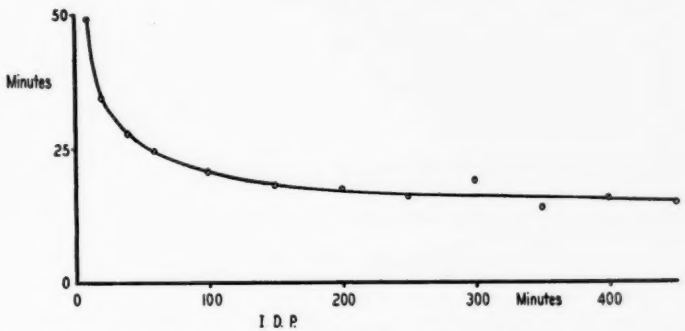

FIG. 4.—Relation between time of application (i.d.p.) of a low auxin concentration (0.03 mg./l.) and time at which growth reaction begins. Each point is an average of 30–100 plants, and is for the single decapitation method.

ture obtained in the standard *Avena* test. This positive curvature may become as great as 2° if blocks are put on very soon after decapitation, and decreases to 0° as the i.d.p. increases to 100 minutes (fig. 3).

2. The *Avena* test curvature (away from the block) begins at about 50 minutes after putting on if the i.d.p. is short. With increasing i.d.p. this period decreases to a limit of about 17 minutes at an i.d.p. of 100 minutes or greater (fig. 4).

3. If the i.d.p. is less than 50 minutes, the auxin curvature shows a regeneration reaction (regression of curvature, figure 3), which occurs at the regeneration time, 130 minutes after decapitation. (Al-

though regeneration time is usually given as 150 minutes, if the regression of curvature is taken to be the regeneration time, in these experiments regeneration time is 130 minutes after decapitation; factors affecting regeneration time are described below; III D_3, III E_5, IV 3a.) For i.d.p. of greater than 50 minutes there is also a regeneration-like reaction which occurs at 80 minutes after applying the auxin (fig. 5). This pseudo-regeneration reaction will be considered later (III A7cd; III D_3; III E_5).

4. The sensitivity of the seedlings to low auxin concentrations increases as the i.d.p. increases, rapidly for the first 30 minutes, gradually from 30 to 240 minutes, and finally decreases after 240 minutes (fig. 3).

5a. For the first 100 minutes of i.d.p. the curvature has a general procedure as follows: a positive curvature (toward the agar block), a succeeding negative curvature (curvature of the *Avena* test), then a temporary regression of this negative curvature (fig. 3).

5b. At about 100 minutes i.d.p. the negative curvature begins, without a preliminary positive curvature, at 17 minutes after putting on, and continues rapidly until 70 minutes after putting on, then continues less rapidly (fig. 3).

5c. For i.d.p. greater than 100 minutes a strong negative curvature begins at 17 minutes after putting on and continues until 80 minutes after putting on. A temporary regression of this curvature follows with a final gradually increasing negative curvature (fig. 3). With increasing i.d.p. this temporary regression decreases until it becomes almost unnoticeable at 300 minutes, and thereafter increases again (fig. 3).

6. The maximum rate of curvature was plotted (in degrees of curvature per hour) against length of i.d.p. It increased rapidly for the first 100 minutes, increased less rapidly from 100 to 250 minutes, and finally was constant after 250 minutes (fig. 7).

7a. The standard *Avena* test (**13, 14**) has two decapitations an hour apart; after the second decapitation the auxin-agar blocks are put on. According to VAN DER WEIJ, the second decapitation increases the sensitivity of the test plants to auxin because it postpones regeneration of the physiological tip. In order to test this contention, tests with decapitations spaced so that there was one de-

capitation just before putting on and so that the plants never remained for more than two hours without decapitation (three decapitations spaced two hours apart were used by HEYN (4) to get

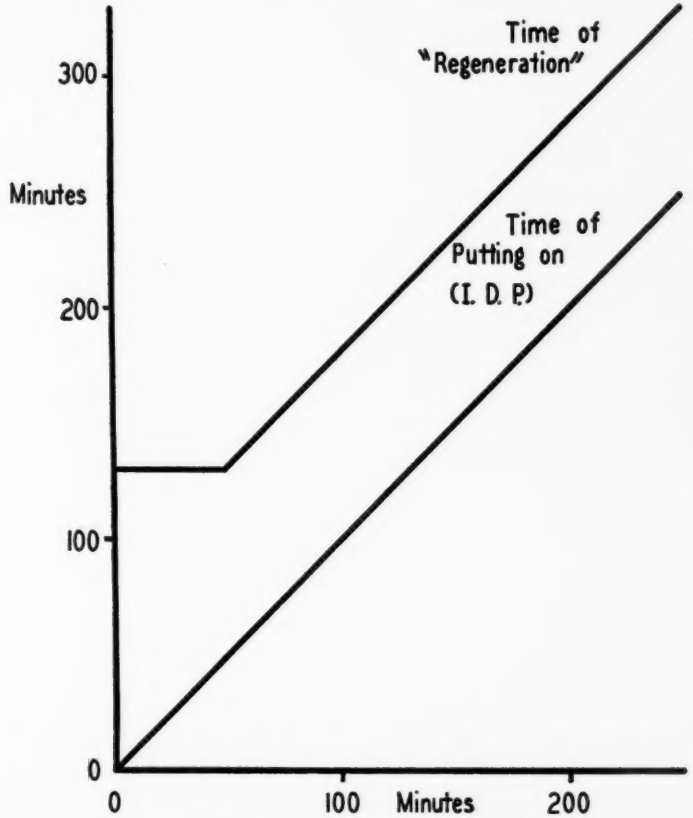

FIG. 5.—Regeneration (regression of curvature) time in relation to i.d.p. for low auxin concentration. Schematic curve from experiments of figure 3.

direct proportionality between auxin and curvature), were run at the same time as the single decapitation tests mentioned previously and with the same i.d.p. as those tests. It was found (for both low and high concentrations) that the first and last decapitations were

by far the most important, so that eventually the intervening ones were omitted.

7b. For each i.d.p. except 100 minutes the procedure of the curvatures was of the same form for both methods. For this auxin concentration, however, curves for i.d.p. greater than 60 minutes showed somewhat greater sensitivity for the two decapitation method (fig. 6).

7c. The two decapitation curve for the 100 minute i.d.p. showed a small regression at about 80 minutes after putting on, while the

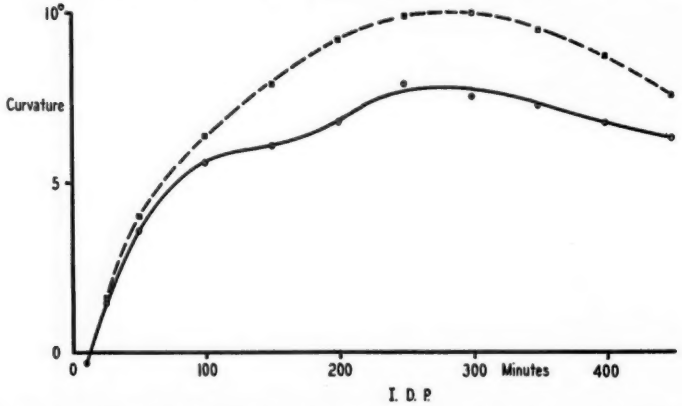

FIG. 6.—Sensitivity of *Avena* to low auxin concentration, 0.03 mg./l., with increasing i.d.p. Solid line for single decapitation (eleven experiments) and broken line for double decapitation method. Measured at 80 minutes after putting on.

single decapitation curve for the same period, as described above (III A5b), showed no such regression. All other curves also showed a somewhat greater regression or tendency toward regression for the two than for the one decapitation method.

7d. On the basis that a second decapitation postpones regeneration (VAN DER WEIJ), it would be expected that for any i.d.p. up to regeneration time, a second decapitation just before putting on would postpone the "regeneration" reaction (regression of curvature) by the length of time between the two decapitations. This was not found to be the case; the reaction was not postponed at all.

7e. The maximum rate of curvature was greater for the two decapitation method than for the single one (fig. 7).

Except for very short i.d.p. (which are generally not used in the *Avena* test), it will be seen from figure 3 that the main curvature for low applied auxin concentrations is completed after about 80 minutes, and after that time may even show a regression. Since this is even more true for the double decapitation method, it is evident that for low auxin concentrations the standard *Avena* test may be ad-

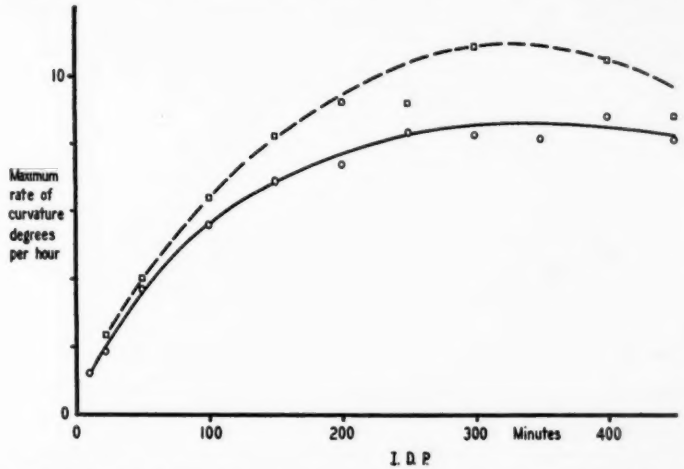

FIG. 7.—Greatest rate of curvature in relation to i.d.p. for low concentration, 0.03 mg./l. Data from same eleven experiments as figure 6. Solid line for single and broken line for double decapitation.

vantageously modified by taking photographs at 80–90 minutes after putting on.

B. THE AVENA TEST FOR HIGH AUXIN CONCENTRATIONS

Figure 8 shows the relation between i.d.p. and procedure of curvature for a high applied auxin concentration giving the so-called maximum angle when the single decapitation method is used. The results are distinctly different from those for low applied auxin concentration (fig. 3).

1. If the i.d.p. is very short, the curvature starts soon after application of the high concentration auxin blocks (about 20 minutes) and proceeds at approximately the same rate for more than three hours.

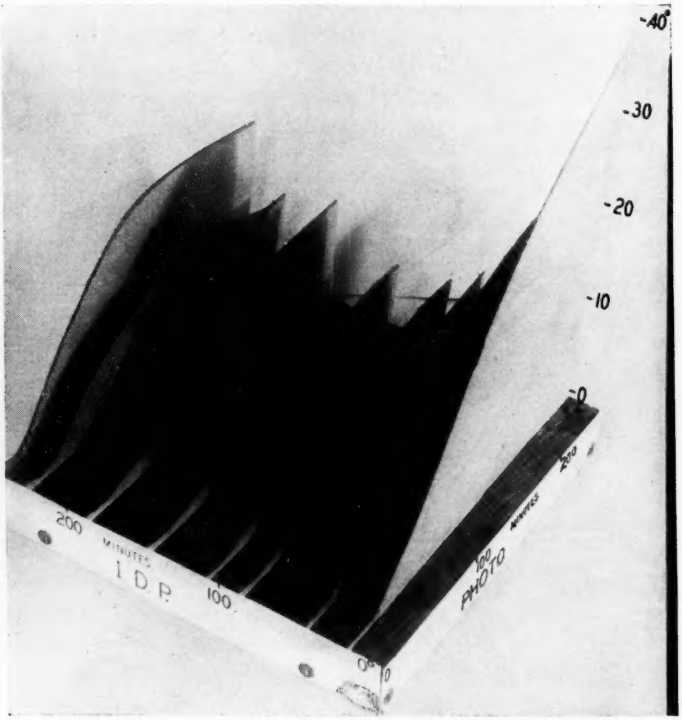

FIG. 8.—Three dimensional model for i.d.p., time of photographing, and curvature, when high auxin concentration (0.5 mg./l.) is applied. Model made from one of three experiments giving same general results. It shows increasing maximum angle with decreasing i.d.p. (for i.d.p. of less than 130 minutes).

2. With increasing i.d.p. the rate of curvature decreases, but the form of the curve is about the same until 130 minutes i.d.p., when a minimum reaction is reached.

3. Beyond 130 minutes i.d.p. the maximum rate of curvature increases again, but the form of the curve is different in that the rate

of curvature decreases sharply or may be slightly reversed at about two hours after putting on.

4. A second decapitation just before putting on greatly increases the curvature with high concentrations (fig. 9); for i.d.p. longer than 130 minutes this increase is about 100 per cent. The concentration

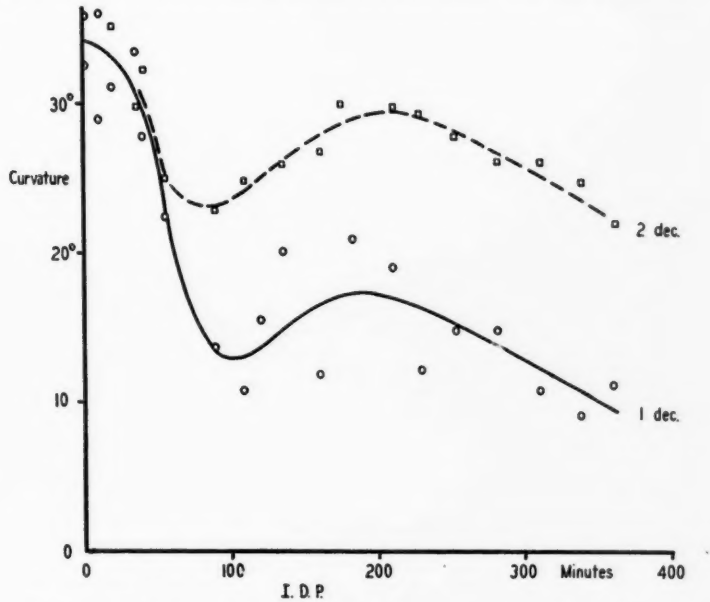

FIG. 9.—One of three similar experiments, showing complex effect of increasing i.d.p. on maximum angle for both single and double decapitation method. For longer i.d.p. double decapitation method gives greater uniformity of results and about twice as high a response.

giving maximum angle is nearly the same for the one and two decapitation methods (fig. 11).

5. To test the explanation that has been given for this increase in sensitivity after a second decapitation (**13**), namely, that the plant is more nearly empty of auxin because regeneration has been postponed by the second decapitation, the following experiments were conducted.

Seedlings were decapitated at three hours after a first decapita-

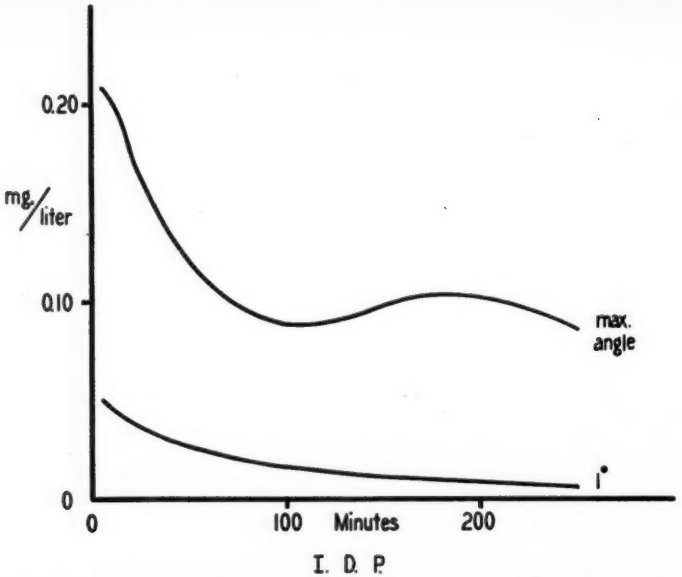

FIG. 10.—Range of concentrations over which proportionality is obtained with increasing i.d.p. Upper curve shows lowest concentration that gives maximum angle at any particular i.d.p., and lower curve shows concentration giving 1° of curvature.

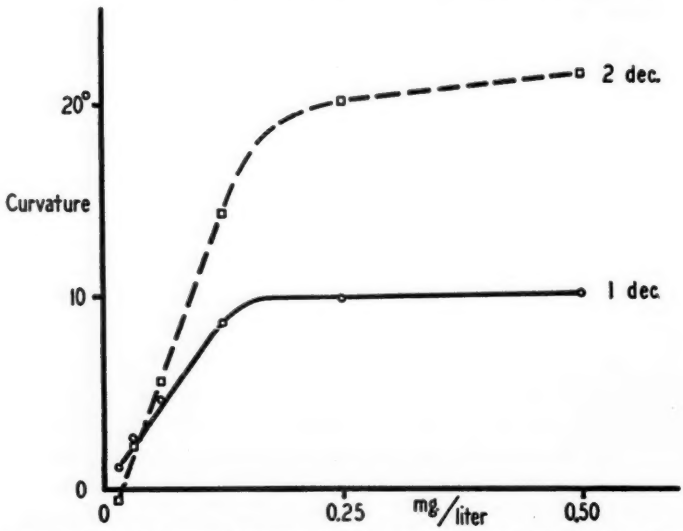

FIG. 11.—Effect of second decapitation just before putting on (broken line) on direct proportionality of curvature to applied concentration of auxin (i.d.p., 150 minutes, photographed at 80 minutes).

tion and a maximum angle concentration of auxin-agar was applied symmetrically to the cut surfaces of two sets of seedlings, one of which was decapitated a second time, the other not. Two marked 5 mm. zones below the cut surface (giving a total of 10 mm., most of the zone over which curvatures occur within two hours) were measured by means of a horizontal microscope to determine growth. Both sets gave the same growth (table 1). Controls without applied

TABLE 1

DIRECT GROWTH MEASUREMENTS SHOWING THAT SECOND DECAPITATION JUST BEFORE PUTTING ON DOES NOT CHANGE MAXIMUM GROWTH AFTER AUXIN APPLICATION BUT CHANGES THE RESPONSE TO AUXIN. FIGURES ARE AVERAGES OF TEN PLANTS

TREATMENT	ZONE	GROWTH IN MM.	
		ONE DECAPITATION	TWO DECAPITATIONS
Auxin-agar (0.25 mg./l.) symmetrically applied	First 5 mm. zone	0.60	0.55
	Second 5 mm. zone	0.55	0.60
	Top zone of 10 mm. (total)	1.15	1.15
Controls without applied auxin or agar	First 5 mm. zone	0.35	0.15
	Second 5 mm. zone	0.45	0.25
	Top zone of 10 mm. (total)	0.80	0.40
Effect of auxin on first 10 mm. zone.....		0.35	0.75

auxin or agar were also measured at the same time and gave the results seen in the table.

From table 1 it is clear that for the one decapitation method the greatest growth difference that can be obtained on the two sides of a coleoptile when maximum angle concentration auxin is applied to one side is 0.35 mm., because the regenerated tip is producing auxin on the opposite side also. For two decapitations this difference is 0.75 mm., since the second decapitation stops regeneration and leaves the side opposite the agar block with only its residual auxin.

The relative difference of the two sides is about 1 to 2, the same as found for maximum angle.

It might be predicted further that since concentrations above 0.25 mg./l. (maximum angle) all give maximum angle, they should also give the same growth as the concentration 0.25 mg./l. Table 2 shows that they do not. DU BUY and NUERNBERGK (1) have ex-

TABLE 2

DIRECT GROWTH MEASUREMENTS SHOWING THAT SUPRA-MAXIMUM ANGLE CONCENTRATION GIVES SOMEWHAT GREATER GROWTH THAN MAXIMUM ANGLE CONCENTRATION. FIGURES ARE AVERAGES OF TEN PLANTS

CONCENTRATION OF AUXIN IN AGAR SYMMETRICALLY APPLIED		GROWTH IN MM.	
		ONE DECAPITATION	TWO DECAPITATIONS
0.25 mg./l.	First 5 mm. zone	0.60	0.60
	Second 5 mm. zone	0.65	0.55
	Top zone of 10 mm. (total)	1.25	1.15
0.75 mg./l.	First 5 mm. zone	0.75	0.70
	Second 5 mm. zone	0.75	0.75
	Top zone of 10 mm. (total)	1.50	1.55

plained this effect by lateral transport of auxin with such high concentrations.

C. PROPORTIONALITY BETWEEN AUXIN CONCENTRATION AND CURVATURE

In view of the very different behavior of the test plants to low and high auxin concentrations when the period between decapitation and putting on of the agar block increases from a few minutes to several hours, the intermediate concentrations had to be investigated as well. This meant an investigation of the relation between concentration and curvature for different i.d.p.

Six experiments were conducted at different times of the year, all giving the same results. A series of auxin concentrations, covering

the whole range between just measurable activity and maximum angle, were put on at increasing i.d.p. (fig. 12).

1. At all i.d.p. the relation between curvature and concentration is a straight line for concentrations below maximum angle.

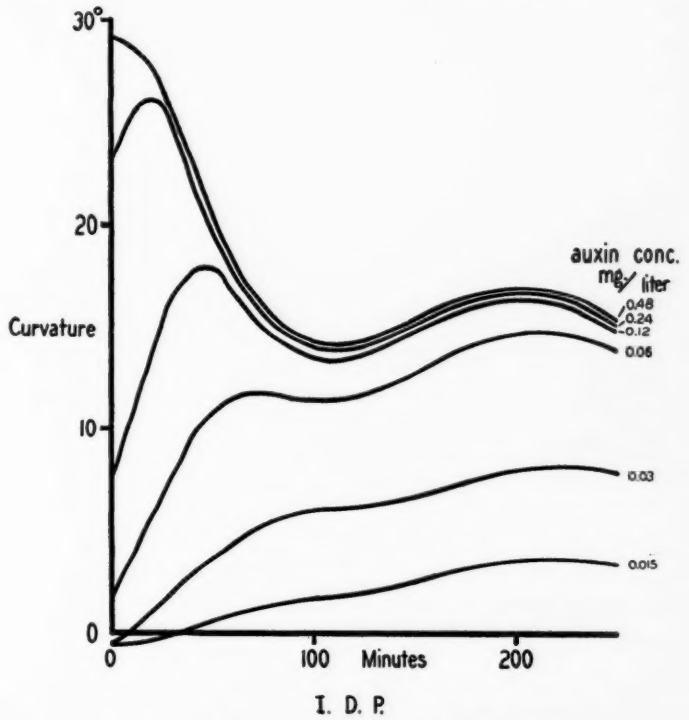

FIG. 12.—Relationship of curvature and i.d.p. for different auxin concentrations and a single decapitation. Schematic curves drawn from combined results of many experiments. Measured at 80 minutes after putting on.

2. For short i.d.p., however, the straight line transects the abscissa to the right of the origin. With increasing i.d.p. the line transects the abscissa nearer to the origin, until after 2–3 hours (this period varies from day to day) direct proportionality between curvature and concentration obtains.

3. For long i.d.p. (3–4 hours), the test plants show a maximum sensitivity (fig. 6), but the range of concentrations that can be determined quantitatively is shifted and is narrower than for short i.d.p. This can be seen by referring to figure 10, which shows that both the maximum and the minimum concentrations which can be tested are lowered by increasing i.d.p.

4. Figure 12 shows that, within limits, the sensitivity for all concentrations of auxin increases with increasing i.d.p.

5. Blocks 1 mm.^{3} and 7 mm.^{3} were used in the *Avena* test to see whether the size of blocks affected the direct proportionality of curvature to applied auxin concentration. Both sizes gave the same curvatures if photographs were taken at 80 minutes after putting on, but if they were taken at 120 minutes the 7 mm.^{3} blocks gave a somewhat greater response that the 1 mm.^{3} blocks. For both cases the curvatures were proportional to the applied auxin concentration, and became more nearly directly proportional as the i.d.p. increased, finally reaching direct proportionality at an i.d.p. of about three hours.

6. Comparison of the one and two decapitation methods over a range of concentrations (long i.d.p.) shows that both give a straight line curve (fig. 11), the two decapitation method giving higher angles for high concentrations and slightly lower angles for extremely low concentrations. This results in the one decapitation method giving more nearly direct proportionality, although both methods approach it closely for i.d.p. of about three hours. For extremely low concentrations (0.01 mg./l.), with a long i.d.p. (three hours), the two decapitation method has a tendency toward positive angles. Under these conditions the two decapitation method gives values 1.5° to 2° lower than the single decapitation method. This is just the amount of the positive curvature which precedes the negative one if auxin-agar is applied immediately after decapitation (III A 1).

It should be pointed out that for very low concentrations the single decapitation method 3–4 hour i.d.p. is best. For moderate concentrations the double decapitation method and 3–4 hour i.d.p. works best because it gives higher angles (which can be measured more accurately) and gives more uniform results.

7. Since (1) the moment at which the plants start to curve is independent of the applied auxin concentration, (2) the curvature is

proportional with the concentration at 80 or 90 minutes after application, and (3) the rate of curvature is constant for about an hour, it follows that at any time after curvature has set in it is proportional with the concentration (for example, see **17**, figure 21).

D. Effect of length of tip removed

Among the other factors affecting the *Avena* test, the length of the tip which is cut off is important.

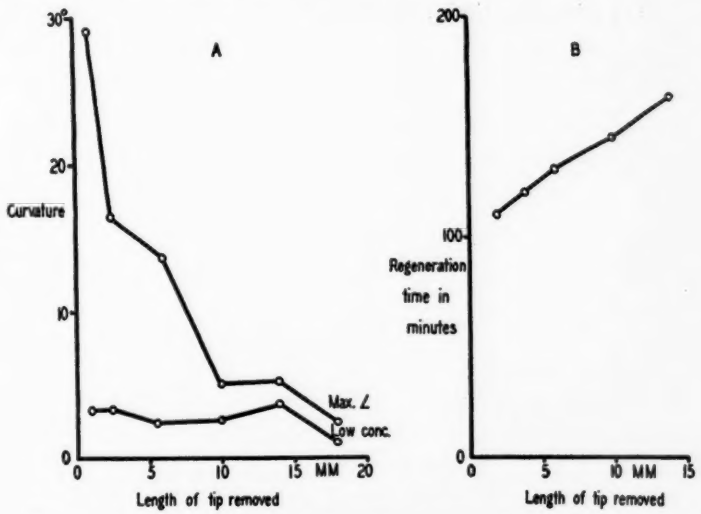

Fig. 13.—(*a*) Effect on maximum angle and low concentration curvatures caused by removing increasingly longer lengths of tip when decapitating. (*b*) Effect on regeneration (regression of curvature) time caused by removing increasingly longer lengths of tip at decapitation. Data are from one of two experiments which gave similar results.

1. As greater lengths of tip are removed, the maximum angle decreases continuously (fig. 13*a*). The rate of curvature is changed, but the procedure of the curvature is otherwise independent of the length of tip removed.

2. With lower concentrations, however, the initial rate of curvature is less dependent on the length of the removed tip (fig. 13*a*), except for old plants where the rate of curvature falls with increasing length of removed tip.

3. With low concentrations it can be seen that the length of removed tip has a great effect on the "regeneration" time; the greater the length removed the longer the period before the curvature starts to recede (fig. 13*b*). This is in agreement with the observations of LI (**6**), who found that the moment of regeneration, as measured by the sudden increase in growth rate of decapitated coleoptiles after the initial gradual drop, is delayed when increasing lengths of tip are removed. Thus, for short i.d.p. it is confirmed that the regeneration of the physiological tip is responsible for the dip in our curves at regeneration time.

E. Flooding with auxin

Since we know that decapitation greatly affects the auxin content of the plants, experiments were carried on in which this content was controlled. To this end a dab of lanolin paste containing auxin was applied to the intact tip for two hours preceding the experiment, thereby increasing the auxin content of the plants above normal. Tests were then run with high and low auxin concentrations.

1. The reaction of low auxin concentrations is decreased compared with untreated plants (fig. 14*a*).

2. More remarkable is the effect on the maximum angle, which first decreases, but with very high auxin concentration again increases (fig. 14*a*). For these high auxin concentrations, however, the curved zone is very short and near the tip. When the auxin concentration was decreased below normal by decapitation and regulated by symmetrical application of auxin-agar to the cut surface, similar results were obtained.

3. There was a greater decrease in sensitivity to low concentrations as the auxin concentration in the plant was increased (fig. 14*b*), than for the paste method above.

4. There was a sharp decrease in sensitivity to high concentration (fig. 14*b*) without the final increase in sensitivity as for the paste method above, probably because the highest concentration applied in the agar was not comparable with the highest applied in the lanolin.

5. The regeneration time increased as the internal auxin concentration increased (fig. 14*c*).

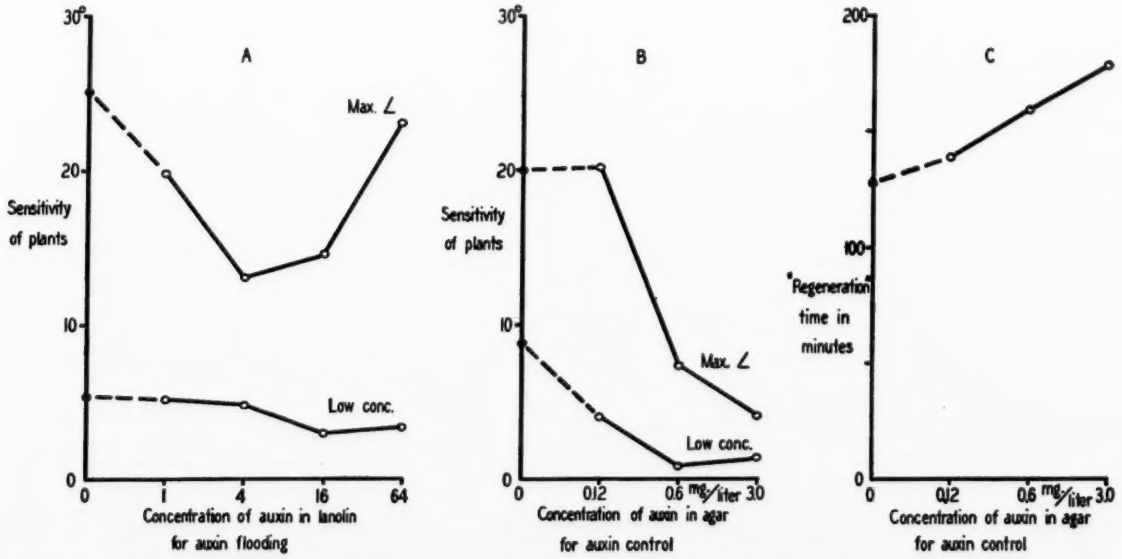

FIG. 14.—(*a*) Effect on maximum angle and low concentration curvatures caused by increasing auxin content (auxin flooding) of intact coleoptiles with lanolin paste. (*b*) Effect on maximum angle and low concentration curvatures caused by changing auxin content of coleoptile by decapitating and symmetrically applying agar and auxin-agar. (*c*) Effect on regeneration (regression of curvature) time caused by changing auxin content of decapitated coleoptile with agar and auxin-agar, both for low and high applied auxin concentrations. Curves drawn from single experiment; a second experiment gave similar results.

IV. Discussion

1. The positive curvature that occurs when agar blocks of low auxin concentrations are put on soon after a first or second decapitation is not yet explained. It exists, however, and cannot be the result of a defective test method because it does not occur when the interval between the last decapitation and putting on is great.

This positive curvature can hardly be caused by loss of auxin into the block because all attempts to get auxin into such blocks resulted only in the obtaining of auxin precursor. It varies in magnitude from day to day, and it is of about the same magnitude for all lengths of tip removed (measurements were made on plants having tips removed varying from 2 to 18 mm.). It seems that the conditions under which these positive curvatures appear are those under which the onset of negative curvature is late.

2. The negative curvatures are of course caused by the influence of auxin on growth, since auxin is a limiting factor for growth in the test plants. Because a test plant having much auxin is already growing at nearly its maximum rate, it is to be expected that additional auxin could not raise the growth rate very much. This is shown to be the case (III E 1–4) where artificially increased auxin content of the test plants lowered the sensitivity to unilaterally applied auxin of low and high concentrations, and (III B 5) where direct growth measurements were made on plants having high and low auxin content by allowing or preventing regeneration. The effect of high auxin paste, however, is an exception not explained in this way.

Since after decapitation the auxin content drops off first rapidly and then more slowly, we should expect that as the i.d.p. increases, the sensitivity to applied auxin would increase first rapidly and then more slowly, which is what has been found for low concentrations (III A 4) and high concentrations (III B 3; III C 4; III B 5).

From this it would be expected that with decreasing i.d.p. the maximum angle would steadily decrease, but for the shorter i.d.p. this decrease is offset by the increased range of sensitivity (figs. 10, 12). Thus the relation between maximum angle and i.d.p. is a com-

plex one, showing two maximums for both the one and two decapitation methods.

3a. From the procedure of the curvatures with low applied auxin, the regression of the curvature for very short i.d.p. must be connected with the regeneration of the physiological tip on the side of the coleoptile opposite the agar block. (The side with the agar block produces less auxin by regeneration because precursor is removed into the agar block, **11**.) Consistent with the explanation that this regression is predominantly a regeneration effect is that for i.d.p. up to 50 minutes it always occurs 130 minutes after decapitation; that is, at regeneration time, regardless of when the auxin is applied (III A 3; fig. 5).

Regeneration is also an important factor in the *Avena* test when high concentrations are applied, and its effects can be eliminated by a second decapitation just before applying the agar blocks, thereby obtaining greater sensitivity to high concentrations (III B 4, 5). There is therefore a regeneration effect on sensitivity and also a regeneration effect on procedure of curvature with low applied auxin concentration and short i.d.p. But we see also that the apparent regeneration effect (regression of curvature) on procedure of curvature with low applied auxin concentration and i.d.p. of longer than 50 minutes must be a result of something other than regeneration.

3b. For i.d.p. longer than 130 minutes, regeneration will have occurred before the agar block was put on and therefore cannot explain the sudden change in rate of curvature at 80 minutes after putting blocks on. There are some other possible explanations of these changes in rate of curvature: (1) Geotropism can be ruled out because the break in the curve occurs even with angles too small to produce geotropic perception in the periods involved, even in intact plants. (2) Aging is ruled out by the suddenness of the break and by the increase in sensitivity for longer i.d.p. which could not occur if aging were a significant factor. (3) This leaves as a third possible explanation, which may be tentatively suggested, the food factor and its transport under the influence of auxin. It has been shown (**16**) that no satisfactory explanation of the effects of auxin can be

given without considering the relation of auxin to other growth factors, in this case the food factor. The food factor moves upward in the coleoptile, perhaps under the influence of auxin, and also seems to be used up during growth. When auxin induces growth in the coleoptile the food factor will be used up so that eventually food factor must limit growth, and thereby limit the rate of curvature. This is similar to the explanation of autotropism by DOLK (2).

V. Summary

1. With an automatic photokymograph, the procedure of curvature in the *Avena* test was investigated. In general, when auxin was applied unilaterally there was little or no effect for about 20 minutes, after which curvature set in and continued at a constant rate for about an hour, then diminished or reversed, depending on the treatment.

2. It was found that increasing the auxin concentration inside the plant decreased the sensitivity to unilaterally applied auxin. This explains the increase in sensitivity after decapitation, since the auxin content decreases after removal of the tip.

3. To study this process in detail, the effect of the i.d.p. (length of interval between decapitation and putting on of the agar block) and the number of decapitations was followed for different concentrations of auxin.

4. For all concentrations up to maximum angle concentration, the sensitivity increased rapidly for the first 30 minutes i.d.p., increased gradually until 200–240 minutes i.d.p., then gradually decreased.

5. But the maximum angle concentration fell off rapidly for the first 100 minutes i.d.p.

6. For i.d.p. longer than 100 minutes, a second decapitation just before putting on causes: (a) the reaction to maximum angle concentration to be doubled; (b) the reaction to extremely low concentration to be slightly decreased, even to the extent of sometimes giving small positive angles; (c) more uniform reactions.

7. A modified standard *Avena* test is recommended: (a) an i.d.p. of 3–4 hours; (b) a second decapitation 20–40 minutes before putting on; (c) photographs at 90 minutes after putting on.

8. Besides the regeneration effect which reverses the curvatures at 130 minutes after decapitation for i.d.p. of less than 50 minutes, there is also a pseudo-regeneration effect for longer i.d.p. which also balances or reverses curvature at 80–90 minutes after putting on.

9. This effect, and certain others (decreases of maximum angle concentration with increasing i.d.p., and positive angles if low concentration auxin-agar blocks are put on soon after decapitation) cannot be explained by geotropism, lateral auxin transport, or aging. Thus it is tentatively proposed that they may be due to food factor distribution.

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DEVELOPMENT AND ANATOMY OF PRIMARY STRUCTURES IN THE SEEDLING OF CUCURBITA MAXIMA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 488

A. GERALDINE WHITING

(WITH SIX FIGURES)

Introduction

Since HARTIG (14) discovered the sieve tube and internal phloem in *Cucurbita pepo* in 1854, numerous studies have been made of the anatomy of the Cucurbitaceae. The investigations have usually been incidental to larger comparative surveys with emphasis on ontogeny and phylogeny. They show a haphazard distribution among the different genera, and at the same time fail to indicate the interrelationships of the various phases studied. The present paper gives a more complete description of the development and the histological anatomy of the seedling of a single species.

After the work of HARTIG, VON MOHL (29) made similar observations on the phloem in several plants, including *Cucurbita pepo*. To both investigators the significance of this tissue was admittedly unknown. Later, when the physiological and cellular nature of phloem was better understood, FISCHER (10) made an extensive study of this tissue in the Cucurbitaceae, noting its character and its unusual distribution. BRAEMER (3), studying the drug plants *Bryonia dioica*, *Citrullus colocynthis*, and *Ecballium elaterium*, differed with FISCHER in regard to the interpretation of certain tissues which the latter called phloem and which BRAEMER considered a segmented lactiferous system quite distinct from phloem.

Meanwhile DE BARY (8) originated the term bicollateral for this type of bundle with internal and external phloem. He cited the Cucurbitaceae as the type. This formulation of a term precipitated a controversy as to whether the internal phloem is actually part of the bundle or whether it is independent and the bundle therefore not bicollateral. Various investigators (15, 21, 25, 12, 1, 28, 5, 30, 19)

have affirmed or denied the direct relationship of this inner phloem to the vascular bundle.

Other anatomical aspects of the Cucurbitaceae also have been studied. The root tip was described by JANCZEWSKI (17). The root itself was studied by VAN TIEGHEM (26) and by RUTLEDGE (24). JANCZEWSKI (18) and VAN TIEGHEM with DOULIOT (27) traced the development of lateral roots but disagreed completely in their interpretations. The transition has been reported by various workers. GÉRARD (13) briefly described it in *Cucurbita maxima*. Later DANGEARD (7) gave a short account, agreeing with GÉRARD, but the following year LAMOUNETTE (21) presented a somewhat different interpretation for the same species. RUTLEDGE (24) has given a more detailed description of the transition in this species. Others (10, 30, 19) have mentioned the transition but only as incidental to some other study. The peg specifically has been studied by FLAHAULT (11), NOLL (23), CROCKER, KNIGHT, and ROBERTS (6), and by others.

In the hypocotyl the primary structure of the bundle in general has been described by the investigators involved in the bicollaterality controversy. Although most of their work is based on the upper axis or stem derived from the epicotyl, much of it may be applied to the bundles in the hypocotyl. In addition to those already mentioned, HOLROYD (16) and ZIMMERMANN (31) have referred to primary structures for a number of different genera, including *Cucurbita*. The course of the bundle in the hypocotyl, through the cotyledonary node and into the cotyledons, has been vaguely mentioned by DANGEARD (7) for several genera, not including *Cucurbita*, and by JEAN (19) for *C. pepo*. The relationship of the epicotyl to this lower axis has been overlooked, except for an incorrect interpretation by DANGEARD (7) and a description of the lower two or three internodes of the stem by MANTEUFFEL (22).

Material and methods

In the present study observations were limited to *Cucurbita maxima* Duchesne. This species is probably subtropical and American in origin (9). As listed by CASTETTER and ERWIN (4), many horticultural varieties have been developed; that used for the present work was the winter squash, Blue Hubbard. For a study of the

seedling, several plantings were made for daily collection up to seven or eight days. Older material was collected approximately each week during the first half of the growing season. All the material was grown under ordinary garden conditions except that the bases of the older plants were protected by wire cages against the destructive activity of the squash vine borer, *Meletiltia satyriniformis* Hübner. In fixing the material, a modification of Navashin's solution and a chromacetic solution were used. The material was cut at various thicknesses: at 6 μ for the study of the origin of lateral roots, at 10 μ for transverse sections of young material, at 15 μ for older material. Longitudinal sections were more satisfactory at 20 μ for xylem but at about 10 μ for phloem. Flemming's triple stain was used, also light green and fast green with safranin. Gourley's method for staining and clearing the vascular system, and a slight modification, produced excellent material for tracing the course of the vascular bundles in the various organs of the plant.

Investigation

Seed and seedling

The seed of *Cucurbita maxima* is characteristically dull white, flat, and elongated or broadly oval with a strong marginal rim which is interrupted at the micropylar end. The anatomy of the seed has been described by Barber (**2**) and Kondo (**20**). In planting, the seed lies flat. Under favorable conditions, at the end of two days the root has protruded between the halves of the seed coat and turned at right angles downward in the soil (fig. 1*A*). The growth of the root is rapid; by the next day it may be 3 cm. in length. This same day, the third day (all dates have reference to the time of planting), the peg begins to develop as a small lateral ridge flattened against the hypocotyl and placed in the angle formed by the horizontal hypocotyl and the perpendicular root (fig. 1*B*). In one or two specimens this outgrowth extended farther around the axis. On the fourth day many lateral roots show just below the peg, and the peg itself has enlarged considerably. At the same time the hypocotyl begins to elongate upward but the cotyledons remain horizontally within the seed coat. The latter soon begins to split, because the lower half is firmly held in place by attachment to the lower surface of the hori-

zontal peg and the upper half is being forced away by the upward growth of the arched hypocotyl (fig. 1*C*). Finally the seed coat splits sufficiently and the cotyledons are drawn out. The recurved hypocotyl with the cotyledons breaks above the soil level about the sixth day, following which the seedling soon becomes fully erect.

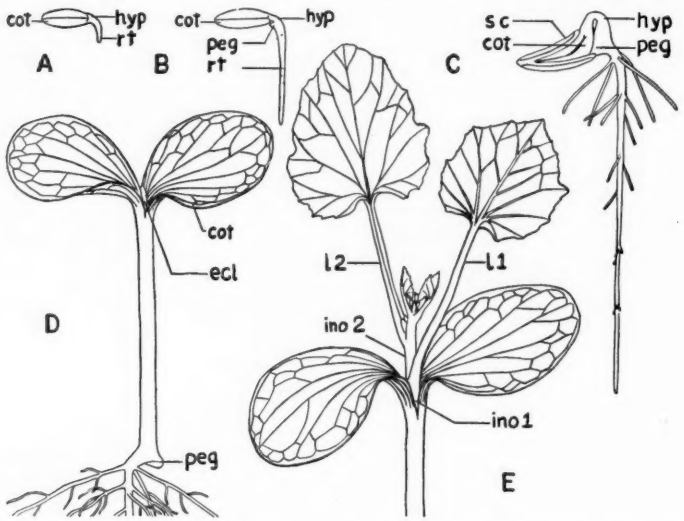

FIG. 1.—Development of seedling: *A*, two days, axis before formation of peg, seed coat removed (*cot*, cotyledon; *hyp*, hypocotyl; *rt*, root); *B*, three days, development of peg; *C*, five days, function of peg in splitting seed coat (*sc*) and withdrawal of cotyledons; *D*, eight days, seedling in which primary growth is completed (*ecl*, epicotyl); *E*, older plant, position of first foliage leaves (*l1* and *l2*) and relative lengths of first (*ino1*) and second (*ino2*) internodes.

The determination of the seedling phase in the life cycle is necessarily somewhat arbitrary. In this case the limit has been placed at about eight days, when the primary structures seem to be completed and most of the stored nutritive material has been utilized so that the young plant is independent and commencing the growth which results in secondary structures (fig. 1*D*). At the end of eight days, then, the plant shows a well branched root system. The primary root is slender, gradually increasing in diameter up to the peg, at

which level the axis enlarges abruptly. This is caused in part by the lateral extension of the peg, and in part by a general enlargement which is maintained through the length of the hypocotyl except for a slight taper below the cotyledons. The hypocotyl is slender and averages about 7.5 cm. in length after elongation is completed. In this variety the surface of the hypocotyl is smooth. The expanded cotyledons are oval, with broad bases, rather thick and green, as is the rest of the plant above the soil. The upper surface of the cotyledon is somewhat hairy but the lower surface is glabrous with prominent veins. The slowly developing epicotyl scarcely shows as yet.

Primary structures

Root tip.—Janczewski (17) classified the root tip of *Cucurbita maxima* as the fourth type. He designated this form as characteristic of only the Cucurbitaceae and the Leguminosae. In this type the identification of several separate histogens is impossible; instead there is one common generative zone from which all tissues arise. In a median longitudinal section of the primary root tip in *C. maxima* this generative zone forms a shallow curve across the tip. At the center the meristem may show about seven layers of cambiform cells, the number diminishing toward the margins. Outwardly this generative zone renews the root cap. At the same time the marginal portions give rise to the dermatogen, the dermatogen and root cap layers dividing from each other so as to effect the "stair-step" appearance in their differentiation, such as Janczewski described for the third type of root. The remainder of the root is derived from cells differentiating inwardly from the central portion of the generative zone. These cells undergo several further divisions which are often irregularly periclinal; then that tissue directly in the center forms the plerome, or stele, and the remaining adjacent cells form the periblem, or cortex.

Root axis.—From this type of apical meristem differentiation of the various tissues is difficult to follow. In the seedlings studied it is further complicated by the rapid elongation of the primary root and the consequent delayed maturation of the tissues. Approximate delimitation of cortex and stele is soon possible, however, because the cells composing the innermost layer of the cortex continue to

undergo tangential divisions for a longer period of time. Within the stele the first tissue to mature is the protophloem. It consists of four arcs of tissue only two or three cells wide, but tangentially extending ten or more cells. In the center of each band, usually wedged between two larger pericyclic cells, a single cell differentiates through changes in its wall and its contents. This cell with its lightly staining contents is conspicuous among the others in which the cytoplasm is much more dense. This protophloem element, although small in diameter, may be considerably elongated. Soon other similar cells differentiate in the band of protophloem; they also are adjacent to the pericycle or may be one or two cells deeper. Among them are other larger parenchymatous cells of the protophloem.

In contrast with the first formed phloem, consisting only of parenchyma, the metaphloem possesses sieve tubes and companion cells. The former are elongated but are small in diameter with relatively small sieve plates. The companion cells are short and have the nucleus and dense cytoplasm usually characteristic of this type of cell. The greater part of the metaphloem is composed of parenchymatous cells, usually larger in diameter than the sieve tubes, variable in length, but generally several times their width. In addition there are some scattered cells with darkly staining and coarsely reticulate cytoplasm.

Paralleling the maturation of the protophloem, the protoxylem has also begun differentiation. The first elements observable are annular with relatively heavy ring thickenings. There are four of these, one at the apex of each protoxylem point. Usually these elements are soon torn, thus initiating the protoxylem lacunae which may later become conspicuous. At the time that this first protoxylem is maturing, the tetrarch exarch pattern of further differentiation is marked out by the successively larger cells in the four primary xylem arms. The two or three cells next within the oldest protoxylem differentiate as delicate annular or spiral elements. The transition from annular to spiral is irregular. Similarly the next two or three elements show a transition in thickening from a delicate spiral to a heavy scalariform pattern. Because of these intermediate types it is not always possible to distinguish protoxylem from metaxylem, but certainly the next vessels form part of the metaxylem. They are

considerably larger than the outer preceding xylem elements and show closely reticulated thickenings. In addition to the elements just described, a variable number of adjacent cells may undergo similar differentiation.

After maturation of these four strands of primary xylem, a considerable area in which further differentiation is retarded remains in the center. It at first has the appearance of pith. This fact may account for VAN TIEGHEM'S (**26**) report of pith in the root, since he studied a young section only 1 cm. from the root tip. GÉRARD (**13**) also noted pith in the root; as he was primarily studying the region of transition, he may have observed a section of the transition at a level in which pith was differentiated, since this tissue descends rather deeply. As RUTLEDGE (**24**) has noted for *Cucurbita maxima* and as seen in the variety used for this study, ultimately two or more cells at the center mature into conspicuously large, pitted vessels (*cf.* fig. 4*A*). The parenchymatous cells surrounding these vessels are isodiametric or horizontally elongated, their walls reticulately thickened and often sinuous. These cells may abut directly against the reticulate vessels in the four earlier formed xylem strands, or two or three layers of vertically elongated, thin walled xylem parenchyma cells may intervene.

Between the primary xylem and phloem a band of tissue remains undifferentiated; this tissue constitutes the cambium. Exterior to these primary vascular tissues is the pericycle. Over the phloem it is composed of a single layer of cells, large in diameter and somewhat elongated. Over the protoxylem points it consists of about four layers of cells, smaller in diameter and only slightly elongated.

During maturation of the stele, the cortex has also matured. At first the innermost layer of cortical cells continues to show tangential divisions, adding to the cortex. At the same time the cells in the outermost layers undergo radial divisions. Finally these different divisions cease, and, with maturation of the scalariform elements the innermost layer becomes the endodermis with narrow Casparian strips. The cells are more elongated and somewhat larger in diameter than those of the pericycle. The parenchymatous cells of the cortex are still larger; they are elongated and show conspicuous intercellular

spaces. Completing the axis is the epidermis, composed of elongated tabular cells many of which form root hairs.

ORIGIN OF SECONDARY ROOTS.—Concerning the origin of secondary roots in the Cucurbitaceae, two radically divergent opinions have been expressed. JANCZEWSKI (**18**) stated that the origin of lateral roots in the Leguminosae and Cucurbitaceae differed from other Phanerogams in that the cortex was involved. In describing the development he declared that the pericycle of the mother root gave rise to the stele of the lateral root, and the endodermis and adjacent cortical layers gave rise to the primary cortex at the surface of which the generative zone developed only later. VAN TIEGHEM and DOULIOT (**27**), in a reinvestigation of this work, rejected JANCZEWSKI'S interpretation. In both *Cucurbita maxima* and *C. pepo* they noted a precise origin of the lateral root from the two pericyclic layers of the mother root, with the endodermis and five or six inner cortical layers forming only a digestive pocket aiding in the outward growth of the young root. The observations made in the present study support the view expressed by JANCZEWSKI.

The primordium of the secondary root originates early in the ontogeny of the primary root, after the differentiation of the protophloem but before that of the protoxylem. In several root tips this showed an initiation of the primordium within 1 mm. of the apical meristem, a longitudinal section in particular showing a primordium 0.8 mm. from the generative zone. The first definite indications of development, as traced in transverse sections, are radial divisions in the two or three inner cortical parenchyma layers opposite the protoxylem point (fig. 2*A*). These divisions are conspicuous in the flatly oval, regularly arranged cells of the cortex. Meanwhile the innermost layer of the cortex continues to exhibit tangential divisions, although in the remainder of the root these are infrequent. At the same time the cells of the pericycle commence dividing rather irregularly, except that the outermost layer, just beneath the tangentially dividing layer of the cortex, also shows tangential divisions. In this way the exact identity of these two layers is soon lost. Longitudinal sections show that the tangential and radial divisions are accompanied by frequent transverse divisions.

Gradually more layers of the cortex become involved in the area

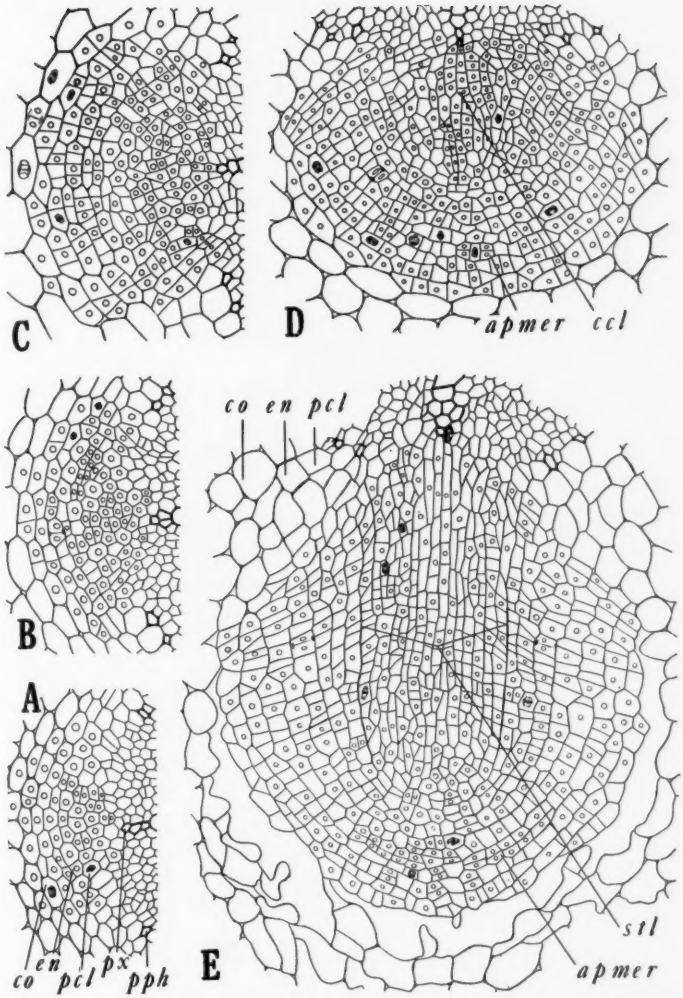

FIG. 2.—Transverse sections from primary root showing origin of secondary root: *A*, first radial divisions in cortex (*co*), and tangential divisions in endodermal (*en*) and outermost pericyclic (*pcl*) layers, nucleated cells in region of primordium (*pph*, protophloem; *px*, protoxylem); *B* and *C*, further divisions in cortex and pericycle; *D*, continued divisions in cortex and pericycle with formation of central cylinder (*ccl*) and origin of apical meristem (*apmer*); *E*, secondary root nearly through cortex of primary root; stele (*stl*) and apical meristem established.

showing radial divisions (fig. 2*B*, *C*). The lateral extent of this zone also increases, the base extending toward the phloem on either side. The cortical cells composing it remain meristematic. They show only a single radial division at first but later several divisions occur, those cells earlier involved showing the greater number. The outline of the mother cell remains traceable, also the layered arrangement of the cortex, although the latter is somewhat distorted by the greater activity and growth of the tissue of the pericycle. In this last region successive divisions produce numerous small, radially elongated cells. With this the lateral root is beginning to take form (fig. 2*D*), the central cylinder of narrow elongated cells forming a stele derived from the pericycle, and curved over this a cap of cortical tissue (and possibly of some pericyclic tissue).

Growth of this primordium continues by further divisions and elongation in the central cylinder. The cortical cap maintains its form and position over the stele by means of successive divisions of those cells nearest the phloem of the parent root. Finally, when all but about three layers of the cortex form part of the cap, a generative zone similar to that already described for the primary root tip develops (fig. 2*D*, *E*). It arises by tangential divisions in approximately the second, third, or fourth outermost layers of the cortically derived part of the root primordium. With growth commencing in this apical area and continuing at the base for a short time, the cortical tissue of the primary root soon tears away around the tip of the primordium, thus freeing the young root to continue growth.

Transition and the peg

Transition.—The early descriptions by Gérard (13) and Dangeard (7) gave the essential idea of the transition; that is, doubling of the number of xylem strands, similar doubling of the phloem, superimposition of these two tissues with reorientation of the xylem from centripetal, through tangential, to centrifugal maturation, resulting in eight bundles separated by narrow medullary rays. Lamounette (21) presented a somewhat different account for *Cucurbita maxima*. He traced the division of the four xylem bundles of the root and the formation of four transition bundles alternate in posi-

tion with the former. Of the four bundles, two divided into two each, forming a total of six which completed the transition and con-

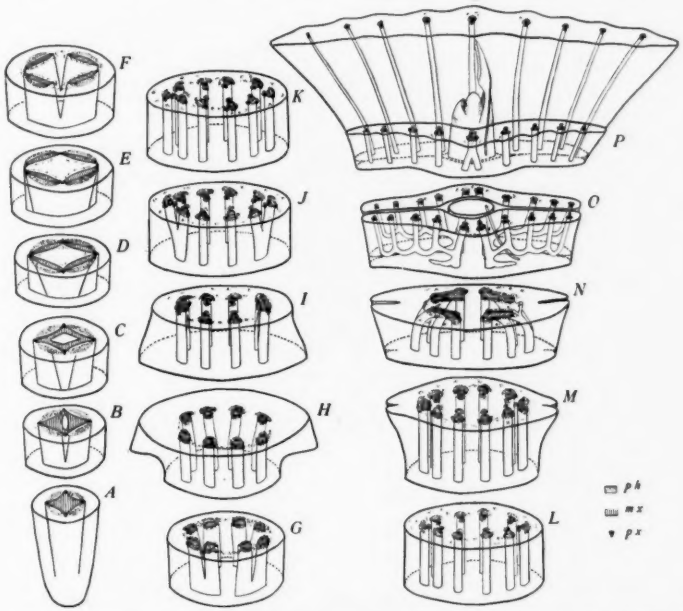

FIG. 3.—Diagram of a seedling, series of transverse sections: *A*, root; *B*, differentiation of pith beginning the transition; *C*, enlargement of pith area with metaxylem in peripheral position; *D*, differentiation of internal phloem; *E*, divergence of each primary xylem strand into two tangential arms; *F*, establishment of dissected siphonostele of four tangential transition bundles; *G*, formation of eight bundles, each with protoxylem still in tangential position; *H*, abrupt enlargement of axis at peg, with protoxylem differentiation becoming endarch; *I*, endarch bundles completing transition, anastomosing of two end pairs of bundles; *J*, end bundles dividing into three to form total of ten bundles which continue through the hypocotyl (*K*, *L*, and *M*); *N*, cotyledonary node, formation of cotyledonary plate by tangential anastomoses, insertion of first foliage leaf trace on dividing end bundle at right; *O*, two median traces to each cotyledon continuing from cotyledonary plate, branching of each trace laterally to establish principal veins in blade of cotyledon; *P*, base of cotyledons, the epicotyl.

tinued through the hypcotyl. Recently RUTLEDGE (**24**) has supported the two earlier writers. The following agrees with RUTLEDGE except for some details in description and interpretation.

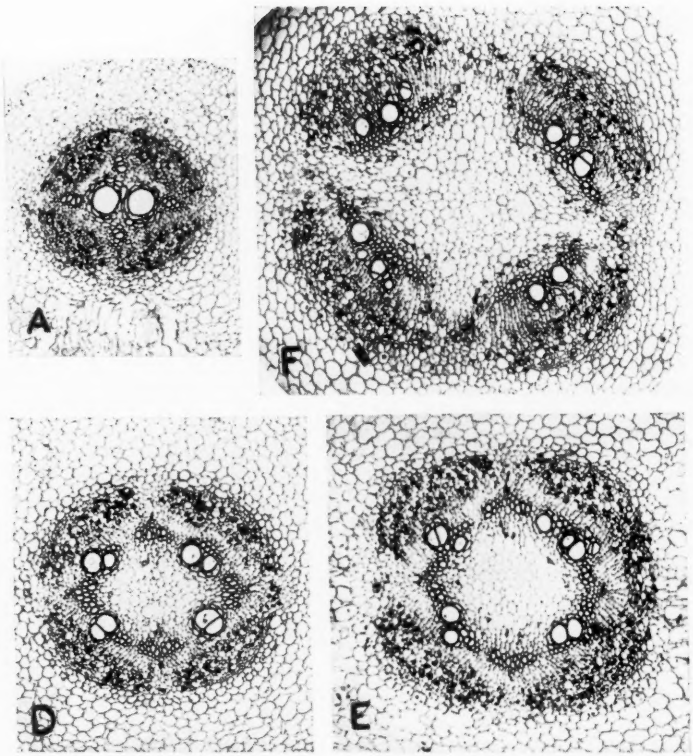

FIG. 4.—Vascular tissues in transition region, series of transverse sections (lettered to correspond with fig. 3): *A*, root just below transition showing differentiation of additional reticulate xylem parenchyma between large metaxylem vessels (*B* and *C* omitted); *D*, central pith and differentiation of internal phloem, identifiable by darkly staining cells within triangular protoxylem strands, metaxylem in peripheral position within external phloem; *E*, divergence of each primary xylem strand into two tangentially extending arms joining the metaxylem; *F*, dissected siphonostele of four transition bundles with tangential band of metaxylem tipped at either end by protoxylem. External phloem masses joined across rays by connective phloem, also phloem along inner face of bundles connected to outer phloem along the rays.

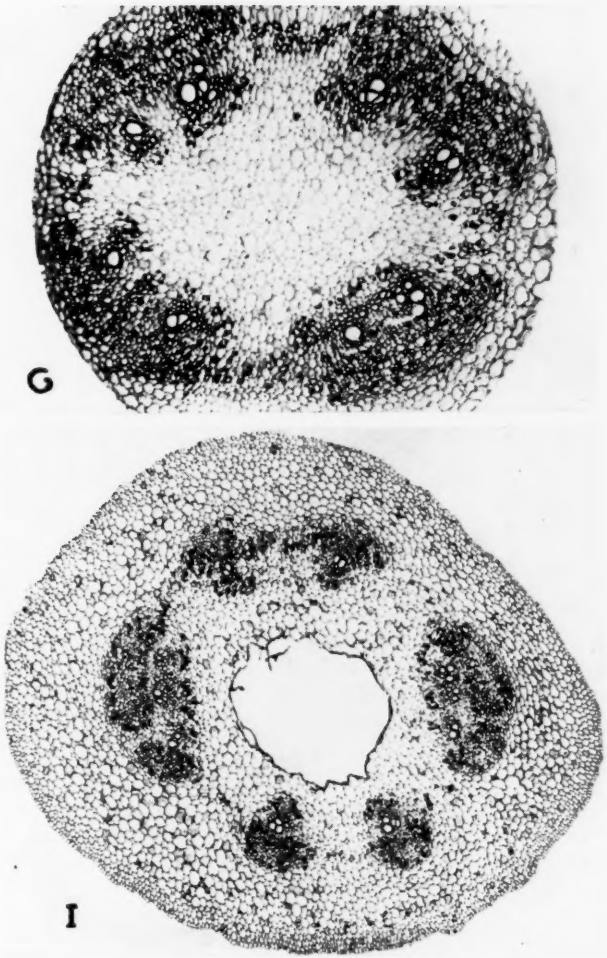

FIG. 5.—Vascular tissues in transition region, transverse sections (lettered to correspond with fig. 3): *G*, eight bundles (one transition bundle just dividing) with phloem differentiating along the new rays, maturation of protoxylem still tangential (*H* omitted); *I*, complete endarch bundles just above peg, also branching of anastomosed end bundle to form the ten vascular bundles of hypocotyl. Differentiation of connective phloem in cortex pericycle, and (less frequently) along the rays.

In *C. maxima* the transition region extends from the exarch protostele in the root somewhat below the peg to the completely endarch dissected siphonostele at a level just above the peg in the stemlike hypocotyl. This involves a portion of the axis over 1 cm. in length in a seedling of about seven days, the exact distance varying with the different specimens. The following description is based on material of that approximate age.

The protostele in proximity to the transition region shows some departure from the structure described as typical of the root (fig. 4*A*). The reticulately thickened xylem parenchyma cells between the large central pitted vessels have increased in number, especially along a median line extending between two opposite protoxylem points. This has separated the pitted vessels into two groups. At the same time these large vessels gradually increase to four or more in number.

The transition, in tracing the course from root to stem, begins where certain of these median cells differentiate as thin walled, vertically elongated pith parenchyma instead of reticulate xylem parenchyma. A narrow band extending from one primary xylem arm to the opposite and in a line at right angles to the plane of the cotyledons results (fig. 3*B*). At higher levels this pith increases in width, continuing to the other two lateral arms of the primary xylem (fig. 3*C*). Concurrent with these changes the pitted vessels by progressively tangential differentiation separate into four groups, which attain positions nearer the periphery of the stele, just within the cambial initials internal to the four phloem groups and alternate with the protoxylem points.

These changes take place gradually, in a spatial relation. Thus about 8 mm. above the wholly rootlike pattern of tissues, the protoxylem and first formed metaxylem still retain the same linear exarch relationship. The inner reticulate tracheae are more numerous, and so broaden the base as to form triangular masses of these primary xylem strands (fig. 4*D*). Connecting these triangles tangentially, and in this way producing a hollow diamond inclosing the pith, is the remaining and later formed metaxylem with the large pitted vessels. By differentiation the number of these vessels again in-

creases from the approximate four to an approximate eight, two in each tangential mass of metaxylem. These vessels are surrounded by reticulate xylem parenchyma which may abut directly against the reticulate tracheae in the triangular masses, or more usually is separated from them by several layers of thin walled xylem parenchyma, as described for the root. External to the metaxylem are the cambial initials, already active at this stage but with no secondary differentiation. Outside this the primary phloem has maintained its original position and relative arrangement. Similarly the pericycle, cortex, and epidermis show continuity with the lower root.

At successive levels above this, reorientation of tissues occurs more rapidly. Because of slight variations in the rate of this reorientation, all four parts of the stele, even though they undergo the same changes, may not show exactly similar structure at any given level. This is especially true of the upper levels. Continuing up the axis 1 or 2 mm., the pith increases in extent and there is a differentiation of internal phloem (figs. 3*D*, 4*D*). Certain cells, frequently those internal to the xylem triangles but several cells removed, undergo divisions producing groups of smaller cells. Among these are some of the conspicuously darkly staining cells already described in the outer phloem. Others are simple phloem parenchyma. A few cells exhibit end walls which are perforated with numerous and somewhat irregularly arranged simple pits. In this way they are similar to the sieve tubes of the outer primary phloem, but the cells are very much larger, and companion cells have not been noted except as the darkly staining cells take this place.

At this level the xylem is not characteristically exarch as in the root. By the maturation of parenchyma in the inner face of the metaxylem immediately subtending the protoxylem, these first formed reticulate tracheae gradually assume a more tangential position in relation to the annular and spiral elements. At successively higher levels each of the original triangular masses becomes divided into two arms spreading toward the tangential bands of the later formed metaxylem. The latter shows the formation of additional small reticulate tracheae which are arranged at first adjacent to the two larger pitted vessels but which progressively differentiate to a position between the two vessels.

Continuing upward, this process of splitting extends to the protoxylem, resulting in irregularly double rows of annular and spiral elements, so that about 1 mm. higher a hollow square of xylem is formed with protoxylem touching at the corners and the sides formed of metaxylem composed mostly of reticulate tracheae, but with about eight separate pitted vessels (figs. 3*E*, 4*E*). Within this square the internal phloem is distributed along all four faces but remains as scattered groups of cells separated from the lignified elements by several layers of parenchymatous cells, some of which may show divisions initiating cambial activity. The parenchymatous pith is composed of large, somewhat elongated cells, circular in cross section, with conspicuous intercellular spaces. The tissues external to the xylem are arranged much as before, except that the lateral extent of the phloem is greater and the rays opposite the protoxylem points are consequently narrower.

In the next 1–2 mm. higher, the pattern of the primary structures is usually complicated by the development of lateral roots at the protoxylem points. Above these, however, each protoxylem point is completely divided into two parts by the differentiation of an intervening parenchymatous ray. As a result, a dissected siphonostele of four tangential bundles is established at this level (figs. 3*F*, 4*F*). Each of these transition bundles consists of phloem and a tangential band of metaxylem tipped at either end by annular and spiral protoxylem elements. The metaxylem forms a continuous layer of small reticulate tracheae with the two or more larger and later matured pitted vessels occupying a position on the outer face of the tangential band. The internal phloem is continuous across the inner face of these bundles but is interrupted at the four primary rays. It is more closely associated with the xylem than at the lower levels. The outer phloem is still directly continuous with that of the root, having maintained both its original arrangement and position. In addition there is frequently differentiation of phloem across the rays connecting the adjacent areas of external phloem, and differentiation in the parenchyma along the side of the ray connecting the inner and outer phloem masses around each bundle.

The diameter of the axis increases more rapidly toward the level of the peg, the pith is larger, and the bundles are separated by

wider rays. At the same time, and about 1 mm. above the establishment of four bundles, the number of bundles increases to eight (figs. 3*G*, 5*G*). This results from the maturation of parenchyma through the center of each tangential bundle. This parenchyma becomes established first in the band of metaxylem interrupting it in the approximate center and so dividing it that each part shows one of the large pitted vessels and other associated tracheae.

Differentiation of the ray through the phloem masses takes place at higher levels, occurring first through the internal phloem and, still higher, through the outer phloem. These eight bundles show characteristics of transition in that the differentiation of the xylem, in passing from the protoxylem to the first formed metaxylem, is still tangential; but the later formation of the large pitted vessels on the outer face of the xylem mass is centrifugal or endarch. The outer phloem retains its relative arrangement except that it now consists of eight masses instead of four. Similarly the internal phloem is present along the inner face of each bundle.

Progressive differentiation from the internal phloem and from the outer phloem in the newly developed parenchymatous ray establishes phloem interconnections along this lateral face just as occurred along the opposite face at a lower level. The internal phloem is separated from the lignified elements by one or two layers of parenchymatous cells which may or may not show cambial activity. Likewise the parenchyma between the lateral phloem and the vascular tissues may show cambial activity. The delimitation of distinct phloem areas is difficult because of this differentiation of phloem across the rays and along the sides of the bundles. Outside the phloem, the parenchymatous pericycle has become irregularly several layered. Next this is the endodermis still traceable as a continuous single layer around the stele, then the parenchymatous cortical tissue, and finally the epidermis which forms root hairs up to the approximate level of the peg.

With the development of the peg the diameter of the axis suddenly increases (fig. 3*H*). In the lower levels this results from increase in cortical tissue on the one side to form the peg. At a level about midway in the peg, however, the stele may also participate in the general widening, the two median bundles on the side toward

the peg curving abruptly outward and the two adjacent bundles also partly sharing in this outward differentiation. Meanwhile, in a distance of about 1.5 mm., at the upper levels of the peg, reorientation of the primary xylem from tangential to centrifugal or endarch differentiation is completed (figs. 3*I*, 5*I*).

Not only does the xylem change in its relative arrangement, but also in the character of the elements composing it. The emphasis on scalariform and reticulate elements characteristic of the root passes to emphasis on spiral or loosely scalariform elements. At the same time there is a gradual decrease in the diameter of the vessels from root to hypocotyl. The phloem shows greater continuity in its character. The outer phloem forms a broad mass capping each bundle, and scattered phloem groups occur across the rays connecting these various outer masses. The internal phloem maintains its position on the inner face of each bundle, but the interconnections with the outer phloem are less frequent. Apparently phloem may also differentiate in the pericycle over the vascular bundles, as the darkly staining cells of the phloem may be found directly adjacent to the endodermis. The endodermis may be traced over the bundles by its small Casparian thickenings, but it is indistinguishable across the rays. At still higher levels it can be identified above the bundles chiefly by its starch containing cells. The remainder of the cortex is composed of large parenchymatous cells with conspicuous intercellular spaces. A cutinized epidermis completes the axis. With the establishment of this dissected siphonostele of endarch bundles the transition is concluded.

The transition in *Cucurbita maxima* is of a fairly simple type. Two features, however, the internal phloem and the peg, add particular interest to it. Differing opinions have been expressed concerning the internal phloem. GÉRARD (13) first described it in the transition region, indicating that this tissue was derived from the outer phloem by inward migration along the rays. LAMOUNETTE (21) denied this, stating that there were no interconnections between the outer and inner phloem, the internal phloem ending blindly below. Further investigation has shown that there is continuity or interconnection of inner and outer phloem, and it is not to be considered that the one developed from the other. Confusion may have arisen

from the fact that the downward extent of the internal phloem seems to vary with the age of the seedling. In a young plant of three or four days it is possible to identify tissue which is internal to the xylem mass of each endarch bundle and which will mature directly into internal phloem. This tissue may be traced to a lower level (approximately fig. 4*G*) where these eight bundles show a tangential arrangement of the xylem, but lower than this it gradually becomes indistinguishable. Progressive downward differentiation takes place so that at about seven days phloem is readily identified at a level where the pith is entirely inclosed by xylem, as described in the transition (fig. 4*D*). Differentiation in the rays seems to correlate with differentiation along the inner face of the bundle. In older stages, therefore, the inner and outer phloem do show interconnections along the rays, but also the internal phloem ends blindly at a still lower level.

PEG.—The peg has presented controversial material in regard to the factors influencing its development, but morphologically the structure is simple. Although the pattern of the transition is already formulated in the embryo, there is no trace of the enlargement which forms the peg. As noted, only on the third day after planting does the peg begin to form as a lateral ridge. Cross sections show that it results from numerous cambial-like divisions in the tissues along a plane extending tangentially across the axis. At lower levels the line of this activity usually involves only the cortex, but at higher levels this plane of divisions may also involve the vascular tissue, which accounts for the abruptly outward course of these bundles.

The broad lower face of the peg is at right angles to the axis, or even at an acute angle; it bears root hairs and often shows traces of reticulately thickened and branched parenchymatous cells which were part of the seed coat to which this surface of the peg was firmly attached during germination. The upper part of the peg gradually merges with the hypocotyl, and shows the smooth epidermis of the latter. CROCKER, KNIGHT, and ROBERTS (**6**) have pointed out that the position of the peg is determined by external influences. Certainly it does not show a constant morphological relationship to the transition. In some cases the greatest dimension of the peg occurs at the level of the four tangential transition bundles, and in other

specimens this level of maximum extent occurs where there are eight or more completely endarch bundles.

HYPOCOTYL

COURSE OF THE BUNDLES.—In the transition from root to hypocotyl a change in the form of the axis occurs, from the circular root to the oval hypocotyl. The peg at the base is on one side of this oval. This form continues up to the divergence of the cotyledons, the longer diameter of the oval parallel to the plane of the cotyledons. Of the eight original bundles in the upper levels of the transition, two are at each end of the oval outline and two along each side (fig. 3*H*). Usually just above the peg and often before the vascular bundles becomes endarch, the number of these bundles increases. This is brought about through anastomosing and branching of the eight bundles, most of these changes taking place within a few millimeters above the peg. Ten was the smallest number of bundles found in the hypocotyls observed; twelve is a common number; and as many as sixteen have been counted. This necessarily involves a wide variety of patterns, the simplest and most frequently recurring of which is described. At the upper levels of the transition the two bundles at each end of the oval anastomose to form a single large bundle which continues up the axis for a short distance before it divides into three (figs. 3*I*, 5*I*). These six with the two along each broad face of the oval make ten vascular strands, the basic number. Frequently an additional bundle is formed on each side of the oval by simple branching of one or the other of the original bundles. This pattern of ten or twelve bundles may continue up the axis to the cotyledonary node (fig. 3*I–M*), or any one of these bundles, especially those at the end of the oval, may give rise to one or more additional bundles. Similarly the anastomosing and divergence at the lower levels may vary considerably; the anastomosing may be omitted and branching may be more frequent. These bundles are arranged in a single ring, in contrast to the two rings characteristic of the upper stem of the Cucurbitaceae.

RUTLEDGE (**24**) described a similar pattern as an exceptional case. He accounts for the increase in number by the formation of additional bundles between the original bundles, frequently between

those on either side of the oval. He does not make it clear as to whether these bundles are independent in origin, or are anastomosed with the adjacent ones. That the latter is the case is shown in material stained and cleared by Gourley's method. The continuity of all parts of the vascular system is evident. It is true that the additional bundles are usually smaller than the others and branching at first involves only phloem, but xylem is later differentiated. Also in certain cases in very young seedlings these additional bundles, after several lateral anastomoses just below the cotyledonary plate, continue for a short time as small strands of phloem which then end blindly.

Contrary to these observations is a statement by DANGEARD (7) that the hypocotyledonary axis in the Cucurbitaceae is later modified by the descent of foliar traces. Since the entire vascular pattern of twelve bundles as described was traced in the procambial strands of an embryo before germination, there is hardly evidence in *Cucurbita maxima* to support this view of DANGEARD.

DIFFERENTIATION IN THE AXIS.—The pattern of the hypocotyl formulated in the embryo consists of tissue showing little differentiation. The epidermis is composed of small closely arranged cells. The parenchyma of the cortex and a similar parenchyma of the stele consist of short, thin walled cells filled with stored nutritive matter and showing characteristic intercellular spaces. Surrounded by this fundamental parenchyma, about halfway between the center and the epidermis, are the provascular strands varying in form and size according to their position in the pattern. They are composed of small elongated cells without the dense contents of the parenchyma cells and without intercellular spaces (fig. 6*A*).

In further differentiation, the first changes occur in the provascular strands. Several elements of the protoxylem mature by the third day. These elements do not differentiate on the inner margin of the procambial tissue, but several cells within it (fig. 6*B*). They are annular and coarsely spiral elements. Unlike the root they are not necessarily adjacent to one another, but may be separated by xylem parenchyma. These first elements are small in diameter, but the later matured elements are larger and the thickening of their walls

is more closely spiral. In the hypocotyl a spiral-scalariform vessel of elongated segments of relatively large diameter seems to be the

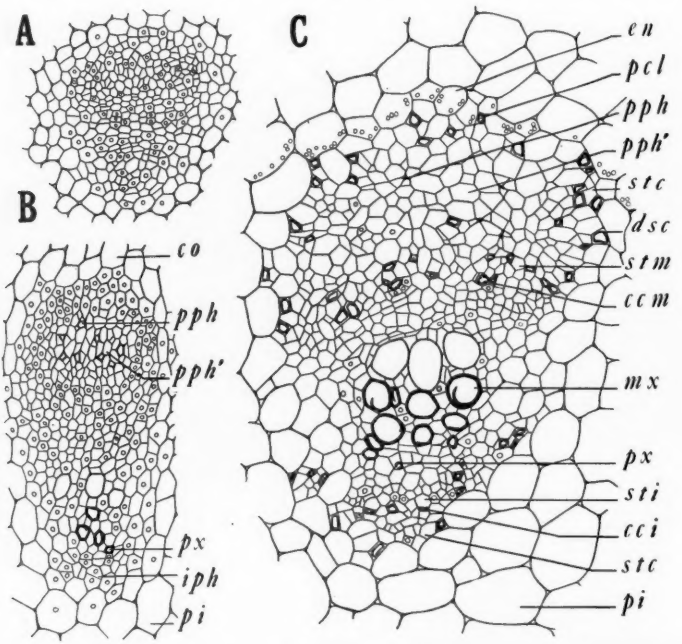

FIG. 6.—Differentiation of vascular bundle in hypocotyl: *A*, provascular strand in embryo just before germination; *B*, vascular bundle of three-day seedling showing outer protophloem with elongated elements (*pph*) and other parenchymatous cells (*pph'*), protoxylem (*px*), and several recently divided cells in undifferentiated internal phloem (*iph*); *C*, vascular bundle of seven-day seedling with primary differentiation nearly completed, showing external phloem with crushed elongated protophloem elements (*pph*) and other protophloem cells (*pph'*) enlarging to become fibers, differentiation of metaphloem with sieve tubes (*stm*) and companion cells (*ccm*), of internal phloem with sieve tubes (*sti*) and companion cells (*cci*), of connective phloem in pericycle (*pcl*) adjacent to endodermis (*en*) and in association with external and internal phloem, chiefly along periphery. Connective phloem conspicuous because of its darkly staining cells (*dsc*); it also shows sieve tubes (*stc*) (*pi*, pith).

end point of primary differentiation, instead of the large, pitted, short segmented trachea such as was found in the root. At the end of primary growth the first protoxylem elements have been torn but

a lacuna is not formed; instead the vessels are closed in by the adjacent enlarging parenchymatous cells.

On the third day the external protophloem is also identifiable (fig. 6*B*). It is similar to that found in the root, with the small, elongated, lightly staining cells interspersed among the larger undifferentiated parenchymatous cells. This protophloem forms a small oval mass of tissue toward the outer face of the bundle. The external metaphloem is much later in differentiation, the sieve tubes and companion cells being first identified in material five days old (fig. 6*C*). In addition the metaphloem shows the darkly staining cells and undifferentiated parenchyma as described for the root. During this later development the small elongated cells of the protophloem are crushed and resorbed. The accompanying parenchymatous cells become enlarged and very elongated. By the eighth day there is scarcely a trace of the crushed cells, and the remaining parenchymatous cells are identifiable as those which later become the lignified phloem fibers characteristic of the hypocotyl (fig. 6*C*).

In the meantime the internal phloem has matured. Since the protoxylem apparently does not form on the inner margin of the provascular strand, one or more layers of procambial tissue remain undifferentiated. There are exceptions to this in some of the smaller additional bundles where the xylem is seemingly formed along the inner margin. The determination of the exact extent of this procambial tissue is impossible. This inner tissue does not show differentiation of protophloem as found in the outer phloem. Instead, during the third and fourth days, cell divisions in these inner layers and adjacent cells increase the extent of this meristematic area (fig. 6*B*). About the fifth day sieve tubes and companion cells are differentiated (fig. 6*C*). Thus the internal phloem corresponds in composition and maturation with the outer metaphloem. Differentiation takes place in those cells toward the pith, and an undifferentiated parenchyma intervenes between the internal phloem and the xylem. It is this zone which later gives rise to the internal cambium but remains inactive during the primary growth.

While the external and internal phloem is maturing, there is also differentiation of phloem strands in non-vascular regions. This unusual distribution was first described by FISCHER (10), who classi-

fied the phloem into several types according to its position: ectocyclic if external to the pericyclic ring of fibers (of the upper stem); entocyclic if within this sclerenchymatous ring; and commissural if connecting these two types and the vascular phloem of the bundles. This classification seems hardly necessary for a study of the hypocotyl. In the first place there is no separation of the ectocyclic and entocyclic systems, but all of these phloem strands form a much branched and interconnected system. So also it is hardly possible to distinguish the commissural phloem. Moreover all this phloem appears structurally similar. On this basis it is simpler to refer to this as connective phloem.

In the primary growth of *Cucurbita maxima* these connective phloem strands appear in the cortical region, in the pericycle, and in the rays around the bundles, although not directly adjacent to the vascular tissue except where the strands occasionally anastomose with either the internal or external phloem. This phloem is abundantly formed in the pericycle. This is especially true over the bundles as noted in the transition region. GÉRARD (**13**) first described this as interruption of the pericycle by the fiber-phloem bundles. Later RUTLEDGE (**24**), finding phloem directly adjacent to the endodermis, concluded that the pericycle was lacking here. But since this phloem is definitely external to the protophloem (fig. 6*C*), it seems more logical to interpret it as differentiation of connective phloem in the pericycle over the bundle.

The connective phloem does not show in the earliest stages of the seedling, but about the fourth or fifth day it begins to develop. This probably accounts for the retarded development of the internal phloem in the transition and similarly along the rays. Certain cells undergo several longitudinal divisions each, resulting in the differentiation of one sieve tube, one or more darkly staining cells, which occupy the position of companion cells, and several parenchymatous cells, all from the one mother cell. In transverse sections of the axis this phloem appears in isolated patches, except where occasional horizontal anastomoses occur. Longitudinally the branched but connected nature of this system is more evident, showing where adjacent mother cells have so differentiated that the sieve tubes are continuous with others vertically or laterally.

Completing the primary maturation of the axis, the other tissues exhibit fewer changes. The epidermis consists of short tabular cells. Stomata are present in the epidermis. Immediately within are several layers of collenchyma. The cortical parenchyma shows large elongated, chlorophyll-containing cells, and the characteristic intercellular spaces have persisted. The endodermis can be traced over the vascular bundles by its conspicuously short cells, which are without Casparian thickenings but contain starch. Across the rays its identity is lost. The pericyclic and pith parenchyma are similar to that of the cortex, except that the inner pith in this variety does not show differentiation of phloem in the primary condition. At the center of the axis a pith lacuna is formed during the later phases of primary growth. This lacuna extends from the upper levels of the transition to just below the cotyledonary node.

Up to this point the description of phloem has followed in general the interpretation given by FISCHER (10); but from certain observations, the question has arisen as to whether there are two types of tissue involved in the phloem. This idea is supported by the work of BRAEMER (3), and a number of his observations parallel those made in *Cucurbita maxima*. The one type of phloem is found only in the vascular bundles, hence can be referred to as fascicular phloem. It is differentiated in both the outer and the inner phloem (fig. 6*C*). In it the sieve plates show a callus formation; the perforations of the plate seem small and regular in arrangement. The cell contents of the sieve tube are homogeneous and rather translucent. The companion cells, two or more to the length of one sieve tube, are filled with dense cytoplasm and clearly show a nucleus. The fascicular phloem is derived from procambial tissue and at the most the sieve tube and the accompanying companion cells originate from the one mother cell.

The second type will be referred to as connective phloem since it is this type only which is found in the non-vascular regions and already has been designated by that name. In addition it is associated with both vascular areas of phloem, but chiefly along the periphery of these (fig. 6*C*). The sieve plates of the connective phloem do not show a callus formation but react to stains as do the adjacent cellulose walls. The perforations in the sieve plates are seemingly larger

and less regular. The sieve tubes may be much shorter than those of the fascicular phloem, and the cell contents show darkly stained, plastid-like bodies scattered through the clear cytoplasm. In this second type of phloem the so-called darkly staining cells are often associated with the sieve tubes as companion cells, but apparently not regularly so. They may be of relatively large size with densely staining, coarsely reticulate cytoplasm and obliterated nuclear outline. In origin this connective phloem appears to be derived from partially differentiated parenchymatous cells. As previously noted, the one mother cell may give rise to a sieve tube, two or more darkly staining cells, and several parenchymatous cells. It is in this connective phloem that BRAEMER found the active constituents of the drugs in the several plants which he studied. Because of this fact, and because it differs so much from the fascicular phloem, he considered it as a segmented lactiferous system. This reinterpretation would eliminate the more unusual aspects of phloem distribution in *C. maxima*. The observations here presented are admittedly limited, however, and suggest only that further work is needed on this subject.

Before concluding the discussion of phloem, reference should be made to the subject of the bicollateral bundle. HÉRAIL (**15**), an early worker on the problem, formulated two criteria for bicollaterality: (a) the derivation of the internal phloem from the procambial strand, and (b) simultaneous development of the inner and outer phloem. He considered that the Cucurbitaceae had definitely bicollateral bundles. The present description of primary differentiation in the bundle of *C. maxima* is in agreement with this; the procambial tissue gives rise to phloem on the inner face of the bundle and the differentiation of this inner phloem is concurrent with that of the outer metaphloem. Later LAMOUNETTE (**21**) described the derivation of the internal phloem from the pith only. His work seems to be on an arbitrary basis inasmuch as it is so often impossible to distinguish between procambial tissue of the vascular strand and the pith. Also the consideration of two types of phloem gives further reason for discarding this latter interpretation. On this basis phloem derived from the pith would be only connective phloem. Actually the vascular type is also found in the inner phloem.

BARANETSKY (1), COL (5), and WORSDELL (30) have rejected these two criteria of HÉRAIL and reinterpreted the bundle from a phylogenetic point of view. They have considered it as not bicollateral but composed of two independent bundles, of which the inner may differentiate only phloem or it may later develop the complementary cambium and xylem. VON FABER (28) found that these potentialities need not in the least destroy the unity of the bicollateral bundle. He reaffirmed the concept of its bicollateral nature on the basis of the ontogeny as he found it for the bundle of *Cucurbita pepo* traced from the apical meristem. The present observations on the bundle in the hypocotyl of *C. maxima* as traced from the embryo agree essentially with the work of VON FABER.

Accepting these criteria with emphasis on the ontogeny of the bundle, that of *C. maxima* is bicollateral. On the other hand, the phylogenetic interpretation is equally important, but the limited scope of the present study provides no answer for this aspect of the question.

COTYLEDONARY NODE

NODE.—At the apex of the hypocotyl the divergence of the cotyledons forms a complicated first node. Like the lower hypocotyl it may show many variations in its vascular pattern, but again can be reduced to a basic form. Tracing upward from the ten vascular bundles previously described in the hypocotyl, the first change in course occurs in those bundles at either end of the oval outline. About 3–4 mm. below the divergence of the cotyledons (fig. 3*N*) the middle bundle at each end divides into two parts, which separate and pass tangentially upward away from each other and toward the adjacent bundles with which they anastomose. These latter continue the tangential course until each end bundle anastomoses with the respectively adjacent central bundle. This reduces the total number of bundles to four, two in the center of each broad side of the hypocotyl. The upward continuations of these four bundles form the two traces to each of the cotyledons. In the course of this general convergence any intervening additional bundles take part. Additional vascular strands between the central bundles on either side of the oval may add considerable variation to the pattern, but ulti-

mately they also anastomose with the adjacent original bundles. The tangential anastomosing produces the effect of four sloping transverse bundles which might be considered the four parts of a cotyledonary plate (fig. 3*N*). Each of these transverse bundles represents essentially the rejoined parts of a single tangential bundle of the transition.

COTYLEDONS.—The four traces just described continue upward and outward into the broad bases of the cotyledons. Before the divergence of the cotyledons is completed, each trace branches laterally, a single vascular bundle differentiating toward the margin of the cotyledon (fig. 3*O*). The course of this bundle is undulating but in general downward. Occasionally there are direct connections between it and that part of the cotyledonary plate immediately below. Four or more large veins diverge upward from this basal lateral vein. The two median traces also continue into the cotyledon; they may anastomose to form one midvein or they may persist independently but with one subordinate to the other. Thus there is established at the base of the cotyledon a complement of at least nine vascular bundles (fig. 3*O*, *P*) which extend into the blade as the principal veins, diverged slightly and interconnected by a network of smaller veins.

From the hypocotyl into the base of the cotyledons the tissues show a simple continuity. The vascular bundles have a structure similar to those described for the axis. They occupy a position at the center of a rather thick mesophyll which is some fifteen or more cells in depth, is composed of parenchyma like that of the cortex in the axis, and shows the same intercellular spaces and connective phloem. On the adaxial surfaces over the vascular bundles there are areas of collenchyma.

The mesophyll in the blade of the cotyledon has a different appearance. In the areas intervening between the large veins the three adaxial layers are closely arranged palisade tissue, and the remaining twelve layers, more or less, form a spongy parenchyma. All this tissue is photosynthetic, although in the unexpanded cotyledon it functioned as a storage tissue. The compact parenchyma is limited to areas adjacent to the more important veins and scattered connective phloem is still found in this. These principal veins are midway

between the two surfaces, but the smaller veins are found interrupting the third palisade layer nearer the adaxial surface. Internal phloem is present in the veins even when only one xylem element is differentiated. The upper epidermis is composed of somewhat larger cells than the lower and it may bear multicellular hairs, but the lower surface is smooth. Stomata appear in both surfaces.

EPICOTYL.—At germination the epicotyl consists of a small growing point overarched by the primordium of the first leaf. In eight days the primordia of six leaves may have differentiated, but the whole structure remains inconspicuous and hidden at the base of and between the two cotyledons (fig. 1*D*). In about two weeks the first leaf has expanded. It is alternate with the cotyledons and is usually found immediately above these, resulting in a short first internode while the internodes next following are much longer (fig. 1*E*). Observation of the embryo before germination reveals that the median trace to the first leaf is already identifiable as a procambial strand although the remainder of the tissue in the epicotyl is undifferentiated. This trace shows correspondingly early differentiation of the xylem elements. Possibly the formation of the short first internode is correlated with this precocious maturation.

Material collected at the end of five weeks, stained, and cleared by Gourley's method showed the vascular pattern of the lower nodes and internodes and the relationship of the epicotyl to the lower part of the plant. Contrary to DANGEARD (**7**), the trace to the first leaf may have the lowest insertion, which is on one or (with branching) on both divergences of the first dividing end bundle of the cotyledonary node (fig. 3*N*). The other bundles of the stem are later differentiated against the lower and more lateral portions of the transverse bundles which form the cotyledonary plate. This produces a leaf gap opposite each cotyledon. The number of bundles and the pattern formed in the first internode are irregular in the several specimens examined. It is only in the second and third internodes that the characteristic two rings of bundles are established. MANTEUFFEL (**22**) has noted a similar variation in the lower nodes of *Cucurbita pepo*. In general his description of the course of bundles in the upper nodes and the divergence of the leaf traces is similar to that found in *C. maxima*.

Summary

1. The root tip of *Cucurbita maxima* possesses a single histogen from which all the primary root tissues arise.

2. The primary root is exarch, tetrarch. Differentiation of the large central metaxylem vessels is retarded; pith is not present.

3. The primordium of a secondary root is formed from the cortex, including the endodermis, as well as the pericycle of the primary root.

4. The transition extends from approximately 1 cm. below the peg to just above it. At the lowest level pith differentiates in the center and the metaxylem takes a peripheral position just within the phloem. Each primary xylem strand diverges into two arms extending laterally and joining the metaxylem. These arms separate, resulting in a siphonostele of four tangential transition bundles. These divide into two parts each, forming a total of eight bundles which become endarch.

5. Of these eight bundles usually two pairs anastomose, then divide into three, producing a total of ten bundles which continue through the hypocotyl. Additional bundles may arise.

6. The bundle is considered bicollateral on the basis of ontogeny; it shows a differentiation of internal phloem from the procambial tissue at the same time that the external metaphloem differentiates. (The study of a single species allows no interpretation on the basis of phylogeny.)

7. A suggestion is made concerning the differentiation of two types of phloem, the one called fascicular phloem and the other called connective phloem. Differences in origin, structure, and distribution of the two types are described.

8. In the cotyledonary node tangential anastomoses produce a cotyledonary plate of four parts. Continuations from these form two traces to each cotyledon. Before the cotyledon diverges completely, each trace branches laterally to form a basal vein from which arise four or more bundles which are the principal veins in the blade of the cotyledon.

9. The bundles of the epicotyl differentiate against the parts of the cotyledonary plate. The epicotyl is retarded in its development

except for the median trace to the first foliage leaf. The early differentiation of this trace may account for the characteristic short first internode.

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LONGEVITY OF SEED AND ESTABLISHMENT OF SEEDLINGS IN SPECIES OF POPULUS

E. H. MOSS

Introduction

Reproduction of poplars under natural conditions in Alberta, Canada, is ordinarily by root suckers and rarely by means of seeds, in spite of abundant seed production in most years and rapid development of seedlings when moisture is supplied to fresh seeds. The present investigation was begun with a view to a clearer understanding of the conditions under which poplars may become established from seed. As set forth in an earlier paper (2), growth of poplars from seeds seemed obviously to depend not only upon climatic and edaphic conditions favorable to the production and establishment of seedlings, but also upon the transfer of seeds in a viable condition. Thus the question naturally arose as to how long the seed will remain viable under various environmental conditions, more especially in the rather dry atmosphere of Alberta. The writer began his investigation without knowledge of the results already published by Japanese workers (3), and with the expectation that subjection of poplar seeds to arid atmospheric conditions would cause rapid loss of vitality.

It has long been known that seeds of salices, while capable of vigorous germination as soon as shed, have only a short period of germinability under ordinary conditions. In comparatively recent years it has been demonstrated that the life of these seeds can be greatly prolonged by appropriate drying at maturity, followed by storage over hygroscopic substances at low temperatures. Certain earlier reports on the prolongation of viability of salices have been summarized by NAKAJIMA (3), who obtained striking results with two species of *Salix*, whose seeds under ordinary conditions retained their vitality for only about one week but under appropriate treatment remained viable for as long as one year. In two species of *Populus* investigated by FAUST (1), longevity is said to be greatly

extended when the seeds are first dried for about five days and then stored in closed bottles at a constant temperature of 5° C. Seeds of *Populus grandidentata* thus treated showed 44 per cent viability after forty-five months; seeds not subjected to any special drying or storage conditions lost their power to germinate after a few weeks. Whereas NAKAJIMA demonstrated the effect of preliminary refrigeration in an icebox followed by storage over hygroscopic substances, FAUST showed the importance of preliminary air-drying and of subsequent storage in closed vessels and at low temperatures. I have been particularly concerned with demonstrating the correlation between various degrees of humidity and longevity and also the combined effect of low humidity and low temperature during storage. In many respects my experimentation and results are similar to those of NAKAJIMA and FAUST, although with one exception the species employed are different.

Experiments on longevity

SPECIES INVESTIGATED AND PHENOLOGICAL DATA

Most of the experiments were with seeds of the aspen, *Populus tremuloides* Michx., the balsam poplar, *P. balsamifera* L., and one of the Russian poplars, *P. petrowskyana* Schneid. A few tests were made on the western cottonwood, *P. sargentii* Dode, and on five species of *Salix*. In all of the experiments, material gathered at Edmonton, Alberta, was used. Since 1931, when the investigation began, the aspen has produced heavy crops of seed in three of the seven years and comparatively little or no seed in the other years. The date of maximum seed shedding for aspen varied greatly from year to year, ranging from May 19 (1931) to June 10 (1935). The flowering dates of aspen for the past twelve years have usually fallen in the third or fourth weeks of April, but range from April 11 (1930) to May 4 (1935), the criterion of flowering being the fully elongated male catkin. Approximately six weeks elapse between anthesis and seed shedding. Records of seed shedding by the balsam and Russian poplars have been kept for the past six years, in all of which an abundance of seed was formed by these species. The balsam poplar begins seed dispersal about two days prior to the Russian poplar and likewise reaches the height of shedding about two

days earlier. Although usually in the third week of June, dispersal has ranged from approximately June 4 (1934) to July 2 (1935). For both balsam and Russian poplars the time between anthesis and maturation of seed has been between forty and forty-seven days. Records of three years for the western cottonwood indicate that this species sheds its seeds at Edmonton during the latter part of July or the early part of August.

Experimental methods

Collections were made when the wool and seeds were emerging from the capsules, only the larger and plumper seeds being used. In the earlier experiments a portion of each collection was placed in a stoppered glass vial at once, another portion was exposed to the atmosphere of the laboratory, and a third portion was placed in a calcium chloride desiccator. After it became apparent that desiccation had the effect, not of reducing but of extending the period of viability, freshly collected seeds were usually allowed to dry for two or three days, exposed to the atmosphere of a dry room at a temperature of approximately 21° C., before being placed under any special storage conditions. This air-drying treatment was somewhat similar to that recently reported by Faust to make for greatest longevity of the seeds, differing mainly in being a day or two shorter and carried out at a temperature usually about 3° lower.

Following air-drying, the seeds were subjected to various storage conditions. A portion of each collection was kept in an open dish, out of direct sunlight, in a room of fairly uniform temperature and humidity. Portions of certain of the collections were stored: in corked bottles at room temperature and at −5° C.; over calcium chloride at room temperature and at −5° C.; over sulphuric acid mixtures designed to provide definite humidities. Details of these various storage conditions are given later.

Seeds were tested for germinability by placing on moist filter paper in petri dishes at room temperature, twenty to forty seeds being used for each test. The usual practice was to record percentage and vigor of germination at the end of two, three, and four day periods. Germination was recorded as follows: (a) "vigorous," when a sturdy hypocotyl bore the cotyledons well aloft within the first two

days; (b) "fair," when the same degree of development required two to three days; (c) "weak," when eventually the hypocotyl elevated the cotyledons well above the filter paper; (d) "no germination," when there was no evidence whatever of life, also when the emerging cotyledons and hypocotyl failed to develop to the degree described as weak. It was found, especially under certain storage treatments, that the seeds retained a sluggish vitality long after power to form normal seedlings was lost.

Results and discussion

Longevity under room conditions.—Room temperature varied from about 18° to 27° C., but nearly always was close to 21° C. The relative humidity was not determined except during one brief period, but available information points to this having been between 40 and 50 per cent during most of the experiments. For the aspen, almost 100 per cent vigorous germination was shown for the first four weeks, after which there was a gradual falling off to 45 per cent vigorous germination at the end of eight weeks. Thereafter viability was lost quickly, dropping to 10 per cent fair and 30 per cent weak germination in twelve weeks, with complete loss of vitality within fifteen weeks. By way of comparison, it may be noted that Faust reports 100 per cent viability at the end of five weeks for aspen seeds stored in open dishes.

Longevity of different salices.—The balsam poplar closely resembles the aspen, while the Russian poplar has a somewhat longer period of viability under the same conditions. The Russian poplar, stored in open dishes in the laboratory, gave 75 per cent vigorous germination at the end of eight weeks, and still showed some vitality, although no true germination, at the end of fifteen weeks. The results for western cottonwood are less reliable, but indicate a period of viability similar to that of the aspen and balsam poplar.

In general, willows apparently have shorter lived seeds than poplars. At the same time, different species of *Salix* probably show wide differences in seed longevity. These conclusions are indicated in Nakajima's review of literature and experimental work, and are given some support by my experiments. *Salix bebbiana* Sarg. showed practically 100 per cent vigorous germination during the first ten

days under room conditions, after which there was a gradual drop to 50 per cent vigorous germination at the end of six weeks, and thereafter a rapid loss in vitality. Thus this willow appears to have a somewhat shorter period of longevity than any of the poplars under experimentation. Four additional species of *Salix* were tested, *S. candida* Flügge, *S. petiolaris* J. E. Smith, *S. planifolia* Pursh., and *S. discolor* Muhl., and all were found to have shorter periods of seed longevity than *S. bebbiana*. Since these species were tested during one season only, and since the last two species gave only 50 per cent germination even when the seeds were still fresh, too much reliance should not be placed upon the following details. In contrast with *S. bebbiana*, which exhibited a sudden falling-off after six weeks, *S. candida* and *S. petiolaris* showed a similar loss in vitality in about three weeks, *S. planifolia* and *S. discolor* in somewhat less than two weeks.

Seeds of the poplar species and of *Salix bebbiana* stored in stoppered bottles remained viable for considerably longer periods than those kept in open bottles or trays. This difference in longevity is probably due in the main to prevention of humidity (vapor pressure) fluctuations in the closed vessels.

HUMIDITY AND LONGEVITY.—Samples of the earlier collections were stored in a calcium chloride desiccator at room temperature. The calcium chloride had been in use for some time in the desiccator and no doubt was hydrated, but the degree of hydration was not determined. Seeds thus stored remained viable much longer than those exposed to the atmosphere of the room.

In order to gain a clearer understanding of the relationship between humidity and longevity, seeds were stored over sulphuric acid solutions designed to provide different degrees of humidity, ranging from extremely dry to very humid. Data on the requisite strength of sulphuric acid required to give definite humidities were obtained from a table published by WILSON (4). In this table, percentages of sulphuric acid are given for relative humidities of 10, 25, 35, 50, 65, 75, and 90 per cent at 0° C. and also at 25° C. By interpolation the writer arrived at percentages of the acid required for the same relative humidities at 20° C., which is close to the mean temperature at which the experiments were run. Then, using the curve for 20° C.,

approximate percentages of sulphuric acid were found for two additional humidities, 83 and 15 per cent; also, by extrapolation of the curve, approximate percentages of the acid were determined for extremely low relative humidities: 5.0, 2.0, 0.5, and zero, the last condition being at least closely approached by use of the concentrated (c.p.) acid. The acid solutions were introduced into wide-mouthed bottles fitted with rubber stoppers, the seeds being held in small cotton bags suspended always at a distance of approximately 3 cm. above the acid. The same volume of acid solution was introduced

TABLE 1

PERCENTAGE OF VIGOROUS GERMINATION OF RUSSIAN AND BALSAM POPLARS, AFTER STORAGE AT DIFFERENT RELATIVE HUMIDITIES

PERCENTAGE		TIME OF STORAGE IN DAYS					
H_2SO_4	RELATIVE HUMIDITY	15	22	39	56	80	127
64.5	10	90	80	90	90	70	25
55.5	25	85	95	85	75	30	0
50.7	35	85	80	80	55	5	0
43.1	50	85	5	0	0	.....	.....
35.8	65	0	0	.....	.....	.....	.....
30.2	75	0	0	.....	.....	.....	.....
64.5	10	80	90	100	90	60	0
55.5	25	75	100	100	65	0	0
50.7	35	75	75	90	45	0	0
43.1	50	45	0	0	0	.....	.....
35.8	65	0	0	.....	.....	.....	.....
30.2	75	0	0	.....	.....	.....	.....

into each bottle, the proportion of liquid to gas volume in the bottle being about one to two.

In the first set of experiments, those of 1935, seeds of balsam and Russian poplars were tested under the humidity conditions shown in table 1. In the second set of experiments, those of 1936, aspen seeds were used and subjected to the more extensive range of humidities shown in table 2. To simplify the presentation, percentages of "vigorous" germination only are included in the tables, percentages of "fair" and "weak" germination being omitted. A certain amount of vitality was regularly exhibited by seeds for some time after power to

germinate, in the sense already defined, had been lost. For example, aspen seeds, stored at 10 per cent relative humidity (table 2), displayed some vitality even after 326 days, but no true germination at that time; although the hypocotyl of a few of these seeds emerged slowly for a short distance, all growth then ceased.

Although the tables show the same general trend for all three species, viability of the aspen is apparently lost at essentially the same rate under the different humidities from 10 to 50 per cent, while viability of the Russian and balsam poplars is retained much

TABLE 2

PERCENTAGE OF VIGOROUS GERMINATION OF ASPEN AFTER STORAGE AT DIFFERENT RELATIVE HUMIDITIES

PERCENTAGE		TIME OF STORAGE IN DAYS									
H_2SO_4	RELATIVE HUMIDITY	2	11	16	25	44	53	65	73	105	326
conc.	0.0	90	85	75	15	0	0	.....	.....	.....	.....
95.0	0.5	100	90	75	20	0	0	.....	.....	.....	.....
85.0	2	100	95	80	30	0	0	.....	.....	.....	.....
75.0	5	100	95	80	50	5	15	0	0	0	.....
64.5	10	100	100	95	90	25	25	10	10	20	0
60.0	15	100	100	100	95	55	50	25	15	10	0
55.5	25	100	100	100	95	55	30	30	20	15	0
50.7	35	100	100	95	95	45	45	35	20	10	0
43.1	50	100	100	95	90	40	30	10	15	10	0
35.8	65	100	100	85	80	5	0	0	.....	.....	.....
30.2	75	100	95	90	65	0	0	.....	.....	.....	.....
27.0	83	95	90	80	10	0	0	.....	.....	.....	.....
18.4	90	40	0	0	.....	.....	.....	.....	.....	.....	.....

longer at 10 per cent than at the higher humidities. The data for aspen are regarded as unreliable in respect to the details already noted, however, and for the following reason. The aspen seeds were collected over a period of four days, and hence different portions had received different degrees of air-drying before being more or less thoroughly mixed together for the experiments; this probably accounts in large part for the somewhat erratic nature of the results for critical stages in the longevity of the seeds. Unfortunately tests were not made between 105 and 326 days, but at the latter time only the seeds stored at 10 per cent relative humidity still retained any

degree of vitality; this indicates a mode of behavior for aspen like that of the other two poplars. Inconsistent aspects of the results shown in table 1 are due to a certain portion of the seeds being nonviable from the beginning.

From these experiments the following conclusions are drawn: (a) Russian poplar has a greater seed longevity than balsam poplar and aspen, a conclusion already reached from earlier experiments. (b) There is a striking correlation between longevity and humidity of the atmosphere during storage. Leaving out of consideration extremely dry conditions (below 10 per cent relative humidity), the drier the atmosphere, the longer the life of the seed, and vice versa. (c) Russian poplar, which under room conditions decreases rapidly in viability after eight weeks, shows a corresponding decline only after eleven weeks when stored in an atmosphere of 10 per cent relative humidity. Balsam poplar and aspen behave in similar fashion. (d) Loss of viability is extremely rapid when seeds are exposed to the more humid atmospheric conditions. To cite an extreme case, aspen seeds after only two days' exposure to a relative humidity of 90 per cent had dropped to 40 per cent vigorous germination, and after eleven days had lost practically all vitality. (e) There is a wide difference between relative humidities of 65 and 50 per cent in their effects upon longevity, as shown by the tabular data. This conclusion has an important bearing upon questions of longevity under natural conditions. (f) The optimum relative humidity for longevity appears to be approximately 10 per cent, for in still drier atmospheres longevity is markedly reduced (table 2). There is a possibility, however, that this reduction in longevity of seeds stored over the stronger acid solutions is due, not to excessive drying, but rather to the toxic action of sulphur trioxide. That there was an appreciable amount of this gas in the atmosphere, at least over concentrated and over 90 per cent sulphuric acid, was evidenced by the turning brown of the cotton bags within forty-four days; by this time the seeds in the bags had lost their vitality.

LONGEVITY AT A LOW TEMPERATURE.—Seeds were placed in a glass vial provided with a stopper and maintained in an electric refrigerator at -5° C. At monthly intervals samples were removed for germination tests. No special care was taken to effect gradual

thawing out of the seeds. Marked extension of longevity was found. Balsam poplar gave 90 per cent vigorous germination after sixteen weeks, and 15 per cent fair germination after thirty weeks. Russian poplar showed 90 per cent vigorous germination after thirty-three weeks and 57 per cent vigorous germination after fifty-two weeks, following which vitality was quickly lost.

The effect of storing seeds in a dry atmosphere at a low temperature was determined. Seeds of Russian poplar were put into a small cotton bag and kept in a large glass vial which was half filled with calcium chloride, the bag being separated from the salt by a thin layer of glass wool. The vial was maintained in the electric refrigerator at $-5°$ C. Remarkable prolongation of longevity was thereby effected. Even after two years seeds thus treated gave 70 per cent vigorous germination.

These results, considered in conjunction with those obtained by NAKAJIMA and FAUST, indicate that seeds of many salices could be maintained so as to have a high percentage of vigorous germination for several years if subjected to appropriate drying and storage conditions.

LONGEVITY UNDER NATURAL CONDITIONS.—In order to determine the effects of certain environmental factors upon longevity, the following experiments were performed.

Seeds were stored on the sill of an east window in such a way as to expose them to fluctuations in atmospheric temperature and humidity without becoming wetted by rain. During the two months, June and July, through the greater part of which this experiment extended, official figures show that the daily relative humidity readings for 5:40 A.M. averaged 77 per cent and for 5:40 P.M. 51 per cent. The mean relative humidity for the period was almost certainly somewhat lower than the average (64 per cent) of these morning and late afternoon records, and probably was well below 60 per cent. Longevity was found to be little more than half that for seeds stored on the laboratory table. Russian poplar, for example, gave a high percentage of vigorous germination for only about four weeks.

To gain some idea of the effect on longevity of alternate wetting and drying, fresh seeds of Russian poplar were dried for two days and then immersed in distilled water for fifteen minutes, after which the

water was drained off and the seeds spread out carefully in a tray to dry. This treatment was repeated at intervals of two days for a twelve day period. In a parallel experiment the period of each soaking was sixty minutes. Germination tests showed that viability of the seeds was soon lost, especially when the period of each wetting was sixty minutes. At the end of twelve days, both lots still germinated fairly well, but thereafter viability was gradually lost. At seventeen days the first lot showed 40 per cent and the second lot 10 per cent fair germination. Five days later there was no genuine germination, although many of the seeds exhibited a sluggish vitality.

From these experiments and those on relative humidity, it may be concluded that longevity of poplar seeds under natural conditions in central Alberta is rather short, usually between two and four weeks, but varying considerably with the species, with the season, and with local environmental conditions. In more humid climates, seed longevity of poplar species would no doubt be considerably shorter than in Alberta.

Establishment of seedlings

Observations and experiments

A number of experiments have been performed in the hope of discovering those climatic conditions in central Alberta favoring the establishment of poplar seedlings. Earlier field observations in the region had shown no evidence of poplars having grown beyond the very early seedling stage under natural conditions. Not infrequently, during a period of damp weather in June, great numbers of poplar seedlings have appeared in gardens and other places, usually in shaded locations, but these have not been observed to live beyond a few days at most.

Growth of seedlings in laboratory.—Earlier attempts at growing poplars from seeds in the garden were unsuccessful even though the seed-bed was protected from direct sunlight and watered regularly. At the same time plants were easily grown indoors, especially when the pots or flats were covered for a few days with glass. It was found that the glass might be removed after three days, without loss of seedlings, even when these were exposed to strong sun-

light from a west window. When glass covers were not used, care had to be taken to add water to the soil frequently, also to avoid exposure to direct sunlight and strong air currents during the first few days. Best results indoors were obtained when the seeds were sown on the surface of sand covered soil, although sowings on compost soil without a sand covering were also successful when proper care was taken in watering.

In short, development and survival of poplar seedlings was found to depend upon the surface layer of the soil being maintained in a moist condition. This cannot easily be realized in a dry atmosphere, except by a close covering, such as is afforded by a glass plate over a flower pot. Success depended also upon watering being done so as to avoid flooding, for the young plants collapsed, often with failure to recover, in a film of water on the soil surface.

MORPHOLOGY OF SEEDLINGS.—The behavior just described appears to be due to an unusual morphological feature of these seedlings, a feature described by YANCHEVSKY (**5**). At the junction of the hypocotyl and root there arises a brush of long delicate hairs whose distal ends adhere to particles in the surface layer of the soil. While the hypocotyl and cotyledons develop rapidly, the root makes very slow growth for several days, the absorptive function being largely performed at first by the brush of hairs. YANCHEVSKY regards the brush of hairs as a successful adaptation because, with a small amount of stored food material, it gives the largest absorbing surface, the greater part of the reserve food being available for the early development of photosynthetic organs. In other respects, however, the brush of hairs cannot be considered adaptive, for seedlings may succumb when the root extends slowly into the soil and when dependence for water supply is chiefly upon this brush of hairs. When the surface layer of the soil becomes dry, the seedling is unable to obtain moisture. It may be too that drying, especially when a soil crust forms, causes mechanical injury to the hairs and hypocotyl, for YANCHEVSKY reports that sand particles adhere so tenaciously to the hairs that it is impossible to separate them without injuring the seedling. Furthermore, flooding the surface of the soil with water may also have the effect of wrenching the seedling from its anchorage and doing irreparable damage.

SEEDLINGS UNDER NATURAL CONDITIONS.—Pots with young seedlings (four to six days in age) of Russian and balsam poplars were transferred from the laboratory to the garden and sunk almost to their full depth in the soil. Different pots were located as follows: (a) in the open, glass covered; (b) in the open, uncovered; (c) in the open, uncovered but protected from direct sunlight; (d) in deep shade, glass covered; (e) in deep shade, uncovered. In one series water was supplied so as to prevent the surface layer of the soil from becoming dry at any time. This involved frequent watering of those fully exposed. In a parallel series, water was supplied less regularly, with the result that the soil in the unprotected pots soon became dry at the surface. The experiments extended over a period of one month, during part of which the heat was extreme.

In the first series there was practically no mortality, except in (a) where all of the plants had collapsed within two days. Here the damage was apparently due in part to the extreme heat below the glass and in part to water drops condensed on the glass, falling upon the plants. Some of the seedlings made poor growth: those in extreme shade apparently because of insufficient light; those in the open because of strong light, extreme heat, and drying winds. Comparatively little damage was done by rain. The most surprising result was the survival of the seedlings in (b), demonstrating that young poplar plants are capable of growth when exposed to extremely hot and dry atmospheric conditions provided the surface layer of the soil continues moist.

In the second series, seedlings in the uncovered pots collapsed sooner or later, the time of collapse coinciding closely with the drying out of the soil in the upper part of the pot.

Here again root development of the seedlings is of interest. A limited number of observations indicate that growth of the root system takes place slowly during the first month. For example, Russian poplars, grown indoors but in fairly strong light, were found at the end of one month to have tap roots averaging only 2.5 cm. in length. It may be added that subsequent development of the root system is much more rapid. By autumn the root systems of the seedlings grown in pots and flats were very extensively branched. By that time too, the plants were capable of enduring rigorous conditions.

Discussion

Establishment of poplar seedlings in the continental climate of central Alberta appears to depend upon the surface layer of the soil being maintained in a moist condition during at least the first week of their growth. Moisture must be available in the upper layer of soil because of the slow growth into the soil of the seedling root, and because absorption at that time is largely dependent upon a brush of delicate hairs near the soil surface. Only rarely in Alberta is the soil surface continuously moist during any considerable period in June and July; although June is generally the wettest month of the year, periods of wet weather are ordinarily short and alternate with periods of low humidity and drying winds. It is conceivable, however, that in the exceptional season—continuous wet weather over a period of one to three weeks—poplar plants may become established from seed.

Finally, an interesting contrast may be noted. The conditions favorable for the establishment of seedlings would be unfavorable towards maintenance of viability of seed. Conversely, the conditions unfavorable for the establishment of seedlings would favor maintenance of seed viability.

Summary

1. The more important results were obtained with the aspen (*Populus tremuloides*), the balsam poplar (*P. balsamifera*), and the Russian poplar (*P. petrowskyana*).

2. Aspen seeds stored in open dishes in the laboratory usually gave 100 per cent vigorous germination at the end of four weeks and 45 per cent vigorous germination at the end of eight weeks, after which viability was quickly lost.

3. Balsam poplar resembles the aspen in longevity of seed, while the Russian poplar has a somewhat longer period of viability under the same conditions.

4. There is a striking correlation between longevity and the humidity of the atmosphere during storage. The optimum relative humidity for longevity appears to be approximately 10 per cent. With increase in relative humidity there is a reduction in longevity.

Loss of viability is extremely rapid when seeds are exposed to the more humid atmospheric conditions.

5. Seeds stored at -5° C. exhibit marked extension of longevity. Seeds stored over calcium chloride at -5° C. show remarkable prolongation of life. Even after two years seeds of Russian poplar thus treated gave 70 per cent vigorous germination.

6. It is concluded that longevity of poplar seeds under natural conditions in central Alberta is from two to four weeks, varying with the species, the season, and local environmental conditions.

7. There is considerable evidence that establishment of poplar seedlings under natural conditions occurs only when the surface layer of the soil is continuously moist during at least the first week of their growth. This is due to the slow growth into the soil of the primary root and also to absorption being largely dependent upon a brush of delicate hairs near the soil surface.

The writer is indebted to Dr. A. W. HENRY for the privilege of using constant temperature refrigerators in the Department of Field Crops, University of Alberta. He is also indebted to the Director of Forestry and to Dr. C. HEIMBURGER, Forest Service of Canada, for an English translation of YANCHEVSKY'S paper.

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A CONTRIBUTION TO THE LIFE HISTORY OF GALINSOGA CILIATA[1]

RICHARD A. POPHAM

(WITH THIRTY FIGURES)

Introduction

According to SMALL, RYDBERG, and a study made by ST. JOHN and WHITE (10), the correct scientific name of the plant with which this paper is concerned is *Galinsoga ciliata* (Raf.) Blake, instead of *G. parviflora* Cav., as given in BRITTON and BROWN or *G. parviflora hispida* DC. as found in GRAY'S manual.

MATERIAL AND METHODS.—Material was collected on the campus of the Eastern Illinois State Teachers College at Charleston, Illinois, during the summers and falls of 1935 and 1936. Hot formalin-acetic acid-alcohol proved to be a better fixative than either corrosive sublimate and acetic acid or Flemming's fluid (stronger solution). The alcohol-toluene-paraffin method of infiltration was used. Entire flower heads were sectioned at 12 μ.

Considerable difficulty was experienced in staining the megagametophyte effectively; Heidenhain's iron-alum haematoxylin intensified with lithium carbonate gave the most satisfactory results. Aqueous safranin counter stained with gentian violet in clove oil gave very good definition in the sporophytes. Clove oil and xylene were employed as clearing agents.

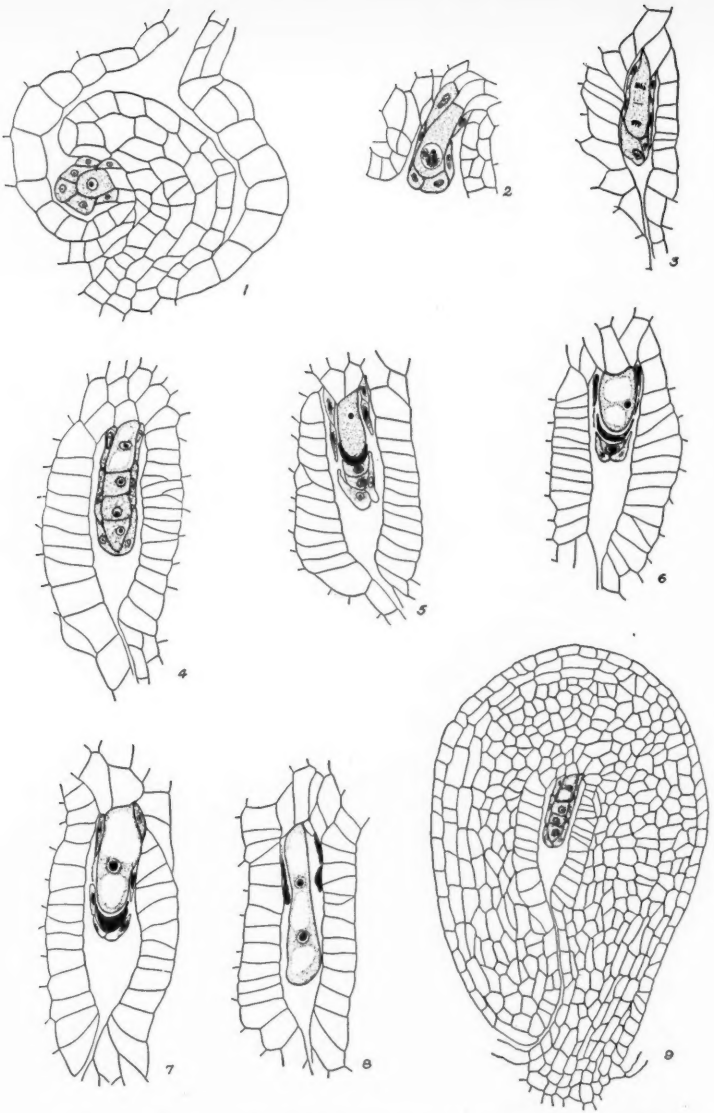
Investigation

MEGASPORE FORMATION

The megasporocyte is hypodermal, one of an axial row of cells. It elongates before the reduction division, and its nucleus, which always lies at the micropylar end, enlarges greatly (figs. 1, 2). No tapetal cells are cut off.

Reduction division (fig. 3) and the second meiotic division occurring successively produce a linear tetrad of megaspores. The micro-

[1] Papers from the Department of Botany, the Ohio State University, no. 391.



FIGS.* 1–9.—Fig. 1, nucellar layer surrounding archesporial cell protruding from young ovule. Fig. 2, elongated megasporocyte surrounded by layer of stretched nucellar cells. Fig. 3, megasporocyte in reduction division; a few integumentary tapetum cells walled off. Fig. 4, tetrad of four megaspores. Figs. 5–7, growth of chalazal megaspore and encroachment on other megaspores. Fig. 8, binucleate gametophyte; remnants of nucellar layer; well defined integumentary tapetum. Fig. 9 (12.5 × ocular), ovule complete in its differentiation.

* All figures are camera lucida drawings using a 15 × ocular and a Zeiss 40 × objective, unless otherwise stated.

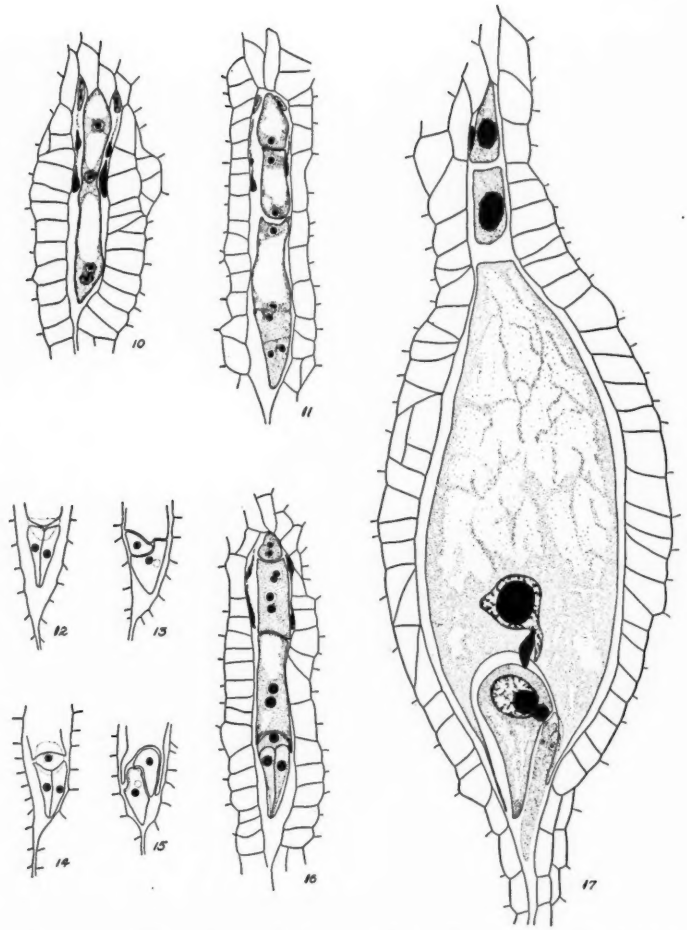
pylar one frequently appears triangular (fig. 4). Normally the cells are uninucleate, but in one case the micropylar megaspore was observed to have two well defined nuclei. The chalazal cell of the tetrad elongates (figs. 4, 5) in the direction of the micropyle. Disintegration of the remaining three megaspores most frequently proceeds from the chalazal to the micropylar end (figs. 5, 6, 7). They finally become compressed into a crescent-shaped body of densely staining material (fig. 7).

The formation of the functional megaspore is the same as reported for *Silphium* (**8**), *Parthenium* (**5**), *Helianthus* (**4**), *Cosmidium* (**13**), *Galinsoga parviflora* (**2**), and *Dahlia* (**9**). In contrast with the usual procedure, LANGLET (**7**) records several tapetal cells being cut off from the archesporial cell before it undergoes reduction division in *Ambrosia maritima*. This may be because *Ambrosia* belongs to a rather distinct group of the Compositae.

MEGAGAMETOPHYTE

The megaspore almost always has large vacuoles, one at the chalazal end of the cell and one at the micropylar end, with the nucleus in a cytoplasmic mass between (figs. 4, 6, 7, 9). When the nucleus divides, the cell elongates from one-half to double its original length and another vacuole, about as large as the original two, appears, separating the two nuclei (fig. 8). After division of the chalazal nucleus the daughter nuclei are separated by another vacuole (fig. 8). One nucleus lies at about the center of the cell and the other in the upper one-fourth, each surrounded by a mass of cytoplasm (fig. 10). After the division of the micropylar nucleus, the two closely associated daughter nuclei and the cytoplasm surrounding them first move to one side of the gametophyte and then drift into the more or less attenuated micropylar end of it, eliminating the original micropylar vacuole and increasing the size of the vacuole formerly separating the nuclei of the binucleate phase (fig. 10).

The vacuoles, cytoplasmic masses, and nuclei are in the same positions in the early eight-nucleate gametophyte as in the four-nucleate stage, definitely showing sister nuclei relationships (fig. 11). The gametophyte remains in the four-nucleate state and also in the mature condition for some time, but the eight-nucleate phase is



FIGS. 10–17.—Fig. 10, four-nucleate gametophyte. Fig. 11, eight-nucleate gametophyte with two antipodals walled off. Fig. 12, micropylar end of gametophyte showing front view of egg and synergids early in differentiation. Fig. 13, side view of similar stage of development as in fig. 12. Fig. 14, slightly later stage than fig. 12. Fig. 15, little later stage than fig. 13. Fig. 16, mature gametophyte with micropylar end in stage advanced over that shown in fig. 14. Fig. 17, megagametophyte at time of fertilization and triple fusion with micropylar end in older stage than shown in fig. 15.

passed through very rapidly. In those mature gametophytes in which the polar nuclei are extremely far apart, indicating that the nuclear divisions have just occurred, the nuclei are almost exactly the same size (fig. 11).

The antipodals are the first to be walled off (fig. 11). The other walls appear almost simultaneously later. The chalazal antipodal cell appears triangular in longitudinal section while the other antipodal is more or less rectangular and usually more than twice as long as the former. In hundreds of preparations, only in two instances were there more than two antipodal cells; three were observed in one and four in another. The chalazal antipodal is originally uninucleate, the nucleus being at the micropylar end of the cell. The rectangular cell contains two of the original antipodal nuclei, one at each end separated by a vacuole (fig. 11). Nuclei of the antipodals occasionally divide (fig. 16). The same procedure of antipodal formation has been reported for other genera (**13, 9, 8**).

Up to date, no other member of the Heliantheae has been shown to have only two antipodal cells with so few nuclei in each. DAHLGREN (**2**) reports three multinucleate antipodals for *Galinsoga parviflora*. TACKHOLM (**13**) reports two antipodal cells in *Cosmos bipinnatus* and *Cosmidium burridgeanum*, but the former may have up to nineteen nuclei in each original cell and the latter commonly develops sixteen to twenty-five.

GOLDFLUS (**3**) has attempted to show that the antipodal cells of many of the Compositae exhibit at times a digestive function and in other species seem to conduct food from the nucellar tissue to the enlarging gametophyte. In *Galinsoga ciliata* the antipodals certainly are not digestive and it is very doubtful whether they are even conductive in function, as evidenced by the fact that disorganization of the nucellus does not begin in the chalazal region but at the micropylar end.

The polar nuclei, separated by a large vacuole, are definitely walled off into a cell originally about the same shape as the micropylar antipodal but at least one-half longer (fig. 16). The two nuclei are almost precisely the same size.

Figure 13 shows a side view of the micropylar end of the gametophyte at the same stage of development as is shown in the front view

(fig. 12). The egg is cut out like a notch, extending about two-thirds of the way into the gametophyte. The walls delimiting the egg shown in figure 13 are in planes running at right angles to the plane of the paper. The wall separating the synergids is in the plane of the paper. The egg and synergids are at first angular (figs. 12, 13) but soon become rounded (figs. 14, 15, 16, 17). The face of the egg exposed to the cavity in which the gametophyte lies elongates toward the micropyle, while the portion of the egg next to the polar cell enlarges (fig. 15). Figures 16 and 17, although not the same stage of development of the gametophyte, show the shapes of the mature egg and synergids in front and side views respectively. Thus the synergids, originally extending almost as far up toward the chalazal end as the egg and occupying all of the micropylar end of the gametophyte, are gradually pushed to one side and are reduced to a comparatively mere fraction of their original size, while the egg enlarges. At maturity the egg is dome-topped, elongated, and pear-shaped (fig. 16). Unlike the synergids in *Parthenium* (**5**), which appear "more or less disorganized and structureless," those of *Galinsoga ciliata* are definitely and clearly walled off.

Just prior to the entrance of the pollen tube into the gametophyte and shortly after the polar nuclei have entered the primary sexual state (**11**) and have slowly moved toward each other and united, the cell containing the polar nuclei increases enormously, both in width and length, and so does the egg. In both the egg and the polar cells, the nuclear membranes become greatly distended and the nucleoli enlarge markedly. The cytoplasm of the polar cell is most concentrated in the end adjacent to the egg, usually leaving numerous vacuoles in the opposite end.

The primary endosperm nucleus has an average diameter of 18 μ, its nucleolus averaging 9 μ. The egg nucleus just prior to fertilization averages 11 μ and its nucleolus 6 μ in diameter. Synergid nuclei vary only slightly from 3.2 μ in diameter, and are at maximum size at their initiation. At this stage of development the gametophyte averages 185 by 47 μ and completely fills the cavity between the funiculus and the integument, as HEGELMAIER (**4**) describes for *Helianthus annuus*.

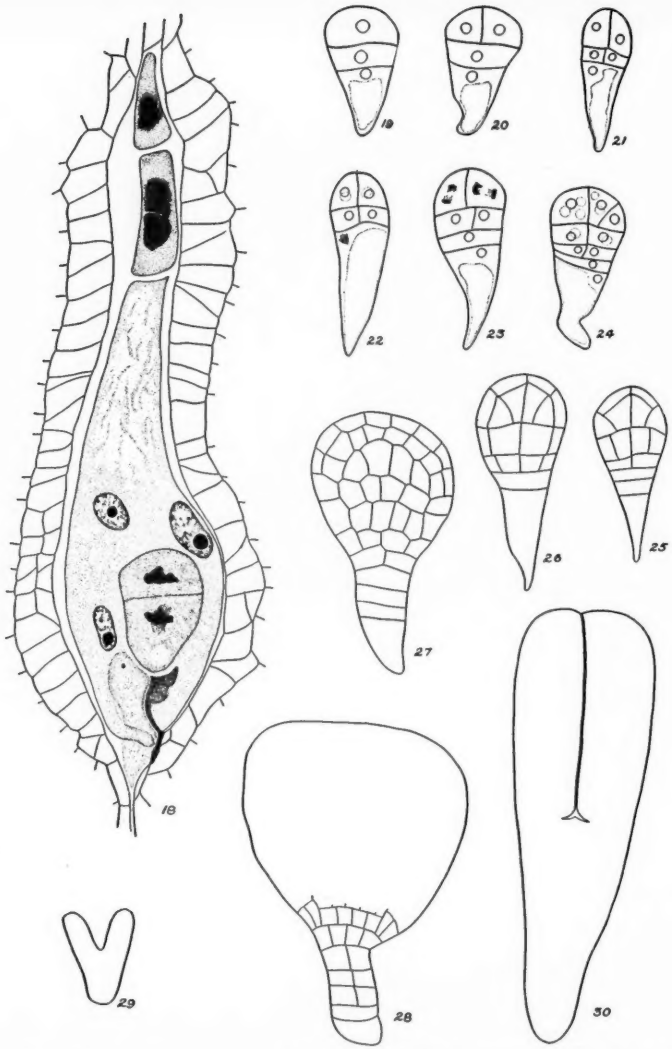
At the time of entrance of the pollen tube through the micropyle,

in most cases the fusion nucleus and the egg nucleus are separated only by the walls of their respective cells. The polar nuclei usually fuse before the pollen tube enters, but frequently both they and the sperm fuse at the same time in true triple fusion, not necessarily "long before fertilization" as in *Silphium* (**6**). There is no evidence that the egg ever develops parthenogenetically. The entrance of the pollen tube may or may not be on the same side of the egg as the synergids, but in any event the synergids usually remain intact. Their nuclei and rather well defined remnants of the cells have been observed in the sac containing the four-celled embryo. The synergids are not consistent in their vacuolations, as MERRILL (**8**) reports for *Silphium*. The pollen tube almost immediately shrivels up but densely staining remnants of it may be observed in sacs having embryos of seven cells. These remnants invariably exhibit the half-moon-shaped opening which may be seen just to the right of the spherical sperm in figure 17.

Usually at fertilization the disintegrating antipodal nuclei of each cell have collected into a central mass of densely staining chromatin material (fig. 17), the walls of the cells still clearly defined. When occasional nuclei that have not disintegrated occur in the antipodal cells, they are longer than usual and of irregular shapes and sizes.

YOUNG SPOROPHYTE

The zygote divides into a hemispherical cell whose derivatives ultimately differentiate into the cotyledons and plumule, and an elongated micropylar cell from which by successive division the hypocotyl and suspensor are developed (fig. 18). The elongated basal cell is the first to be divided, a transverse wall paralleling the original wall (fig. 19). The terminal cell is divided by a wall perpendicular to the first two walls (fig. 20). The cell just below the two apical cells is divided by a wall in the same plane as that dividing them (fig. 21). A seven-celled stage results when a vertical wall divides both apical cells (fig. 22). The basal elongated cell is then divided by a wall parallel to the plane of the original wall which divided the zygote into two cells (fig. 23). Sometimes the basal cell divides before the apical cells. The nuclei of each cell of the apical



FIGS. 18–30.—Fig. 18, gametophyte with two-celled sporophyte, densely stained pollen tube, one synergid, and free nuclear endosperm; spindle in apical cell of sporophyte lying in transverse plane; spindle in micropylar cell lying in plane of paper. Figs. 19–27, successive stages in development of sporophyte. Fig. 28, outline of median section of older sporophyte showing maximum observed development of suspensor. Fig. 29 (15 × ocular and Zeiss 8 × objective), older stage; suspensor probably crushed and disintegrated. Fig. 30 (15 × ocular and Zeiss 8 × objective), sporophyte of mature seed and fruit.

quadrant and the two cells basal to them divide (fig. 24). The basal one is again divided by a transverse wall, while a vertical wall, in the plane perpendicular to the plane of the paper, separates the nuclei in the terminal cell of the basal row (fig. 24). Anticlinal walls divide each cell of the apical quadrant. One such wall is shown in figure 24. The dermatogen is first cut off from the four terminal cells of the apical octant and later from the lower four. Similarly the dermatogen is cut off first in the terminal cells produced by the original basal cell of the two-celled sporophyte and later in the basal ones which have divided longitudinally (figs. 25, 26). In figure 27 the dermatogen layer of the cells originating from the apical hemispherical cell of the two-celled sporophyte has been divided by a second set of periclinal walls as well as by anticlinal ones; all except the dermatogen cells and the basal cell have been cut by longitudinal walls; and the basal one has been divided by three transverse walls. The maximum development of the suspensor observed is shown in figure 28. Figures 29 and 30 are outline drawings (on a much smaller scale) of later stages of sporophyte development. In the specimen from which figure 30 was made, not only the epidermis but the palisade and spongy mesophyll were unmistakably differentiated. No stomata were observed. In later stages of development (figs. 29, 30) no suspensor remnant was found; probably it had been crushed in the rapid enlargement and elongation of the sporophyte. A similar condition exists in *Helianthus annuus* (1).

In a general way, the development of the sporophyte compares with that in *Parthenium* (5) and *Helianthus* (1).

Endosperm

By the time the first division of the zygote is complete, from two to ten endosperm nuclei have been formed. The same is true for *Silphium integrifolium* (8). Dahlgren (2) not only figures a cellular endosperm at the two-celled embryo stage but definitely states that one exists in *Galinsoga parviflora*. Observation of many preparations by the writer and others has given no support to the supposition that a cellular endosperm is found from the beginning in *G. ciliata* (fig. 18). Kirkwood (5) makes no definite statement as to when the endosperm nuclei are walled off in *Parthenium*. The endo-

sperms in *Bidens tripartitus* and *Silphium perfoliatum* (**2**) and *Xanthium* (**12**) are cellular from the beginning; the endosperms of *Helianthus annuus* and *Dahlia coronata* (**9**), *Silphium integrifolium*, *S. terebinthinaceum*, and *S. laciniatum* (**6**) are free nuclear at first, just as in *Galinsoga ciliata*. The first indications of the formation of cell walls which separate the endosperm nuclei may be seen occasionally in sacs containing three-celled embryos, but definitely defined walls were not observed until the six-celled embryo stage. All of the nuclei seem to be walled off simultaneously and the endosperm tissue completely fills the gametophyte. The tissue seems to increase in number of cells up to about the stage of development represented by figure 26, after which time the cells enlarge and disintegrate as the sporophyte enlarges. By the time the sporophyte has reached the size represented in figure 30, no endosperm tissue remains within the tapetal layer. It is not until this time that the sporophyte completely fills the cavity within the tapetal layer.

NUCELLAR LAYER

From the time of the initial enlargement of the megasporocyte, it and the cells developing from it are surrounded by a single layer of nucellar tissue which always appears in longitudinal section to be composed of six cells (figs. 1–9). At first these cells are about the same shape as the rest of the more or less spherical ones of the nucellus (fig. 1), but as the megasporocyte elongates they are stretched, those along the sides being longer than those at the micropylar end (figs. 2, 3); they soon break away from the rest (fig. 5). The nuclei of the upper four cells begin to disintegrate (fig. 5), those of the middle pair usually starting first. The two micropylar ones remain intact longer than any of the others. Similar behavior has been reported for *Silphium* (**8**). At the binucleate stage all of the cells have disintegrated into masses of densely staining material. Occasionally these remnants can be observed in sacs containing two-celled embryos.

Apparently the remnants of the nucellar layer at the micropylar end of the tetrad are pushed aside when the megaspore elongates and becomes binucleate.

From the time the gametophyte is binucleate (fig. 2) to the time of seed maturity, it lies free in the cavity formed by the integument

and the funiculus, being attached to the ovule only at its chalazal end. This same condition is found in *Parthenium* (5) and *Helianthus annuus* (4).

Tapetum

Soon after elongation of the megasporocyte, epidermal integumentary cells bordering the cavity begin to elongate at right angles to the longitudinal axis of the cavity (figs. 2, 3). By the tetrad stage, these cells (ten to fourteen on each side of the cavity in longitudinal section) have become well differentiated (figs. 4, 9). Aside from individual fluctuations, the number of cells is constant up to the time of the great enlargement of the micropylar end of the sac (fig. 16). At that time rapid cell division increases the number to between twenty and twenty-seven (fig. 17). During this period of activity some of the tapetal cells occasionally divide with their spindles at right angles to the axis of the gametophyte, forming in small patches a double layer of narrow tapetal cells (figs. 17, 18). A similar condition has been observed in *Helianthus annuus* (1). Very little if any increase in the number of cells takes place again until about the time the dermatogen is cut off in the embryo (figs. 26, 27). As the latter continues to enlarge from this stage on, the number of cells in the tapetum increases until it is more than fifty cells long on each side in longitudinal section. The cells become stretched so that instead of their longest dimension being at right angles to the long axis of the sac, it is parallel to it.

Ovule

The ovule has only one integument, which is early apparent (fig. 1) and which assumes its ultimate position with reference to the nucellus before the tetrad stage is reached (fig. 9). The nucellus next to the tapetum begins to disorganize when the eight-nucleate gametophyte is formed (fig. 16); and by the time of fertilization, when the sac has enlarged decidedly (fig. 17), a considerable cavity is apparent between the outer layers of the nucellar tissue and the tapetal layer. The same condition was observed by Hegelmaier (4) in *Helianthus*. Disintegration of the nucellus begins and proceeds most rapidly in the region where the greatest enlargement of the gametophyte takes place. This is in striking contrast to *Dahlia gracilis* (3), in which disorganization of the nucellus begins first in the chalazal

region around the antipodals and proceeds only slowly toward the micropyle.

Originally there are a dozen or more layers of cells in the ovulary wall, the inner ones of which are stretched and destroyed by the rapid growth of the ovule. By the time the embryo has reached the stage of development represented in figure 30, only about three to five rows of pericarp remain and the entire ovule tissue except the tapetal layer has disappeared.

NOTE: A short abstract of an unpublished dissertation by C. J. HARRIS on *Galinsoga ciliata* (Raf.) Blake appears in the University of Pittsburgh Bulletin 32: 131–137. 1935. Part of the conclusions in the abstract agree with the findings presented in this paper and part do not. Since the paper has not been published in full and there are no illustrations with the abstract, it was thought best merely to cite the reference.

Summary

1. The archesporial cell of *Galinsoga ciliata* cuts off no tapetal cells but develops directly into the megasporocyte.

2. Reduction division and the second meiotic division result in a linear tetrad.

3. The chalazal megaspore develops into an eight-nucleate, six-celled gametophyte, and the other megaspores disintegrate.

4. The large angular synergids originally extend almost as far up toward the chalazal end of the gametophyte as the small angular egg, and occupy almost all of the micropylar end of the sac. These gradually become rounded, reduced to a mere fraction of their original size, and are pushed to one side by the egg, which soon after its formation rounds up, enlarges, elongates, and occupies almost all of the micropylar end of the gametophyte.

5. The layer of nucellar cells surrounding the megasporocyte and tetrad disintegrates during the development of the megagametophyte.

6. There is an integumentary tapetum.

7. The two antipodals occasionally have as many as four nuclei each.

8. The pollen tube enters the ovule through the micropyle.

9. Triple fusion occurs.

10. Two to ten endosperm nuclei are present when the first division of the zygote is complete.

11. The free nuclei of the endosperm are walled off at about the time the sporophyte becomes six-celled.

12. The apical cell of the two-celled sporophyte gives rise to the cotyledons and plumule.

13. The basal cell of the two-celled sporophyte gives rise to the hypocotyl and suspensor.

14. The mature fruit contains a dicotyledonous sporophyte, no endosperm, the tapetal layer, and a pericarp three to five cells thick.

I am deeply indebted to Dr. ERNEST L. STOVER for suggesting this problem and to Dr. GLENN W. BLAYDES and Professor JOHN H. SCHAFFNER for their valuable counsel and encouragement during the progress of this work.

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EMBRYOGENY OF ACORUS CALAMUS

MURRAY F. BUELL

(WITH NINETEEN FIGURES)

Introduction

Two attempts have been made in Europe to study the embryogeny of *Acorus calamus* L. MÜCKE (**6**) and JÜSSEN (**5**) both found that the megagametophyte in the European plant fails to mature. MÜCKE was able to describe only the very early stages. Considerable emphasis, however, was placed on a study of the perisperm. MÜCKE also discussed the postament and referred to the work of WESTERMAIER (**8**) on *Aconitum*, in which such a structure was first described. He illustrated the mature megagametophyte as well as the seed of *Acorus gramineus* Soland. JÜSSEN traced the development of the megagametophyte of *Acorus calamus* a little further than did MÜCKE, but not beyond a four-nucleate stage.

Investigation

The material for this study was collected in Minnesota, where seeds normally mature (**1**, **3**). Here the spadix is formed by the middle of May, but not until well along toward the end of May are the ovules sufficiently developed to show the primary archesporial cell. By about the middle of June pollination takes place, and by the middle of August the seeds are ripe. A rest period is necessary before germination occurs; even when planted in the greenhouse the seeds do not show activity until February.

ORIGIN OF INTEGUMENTS

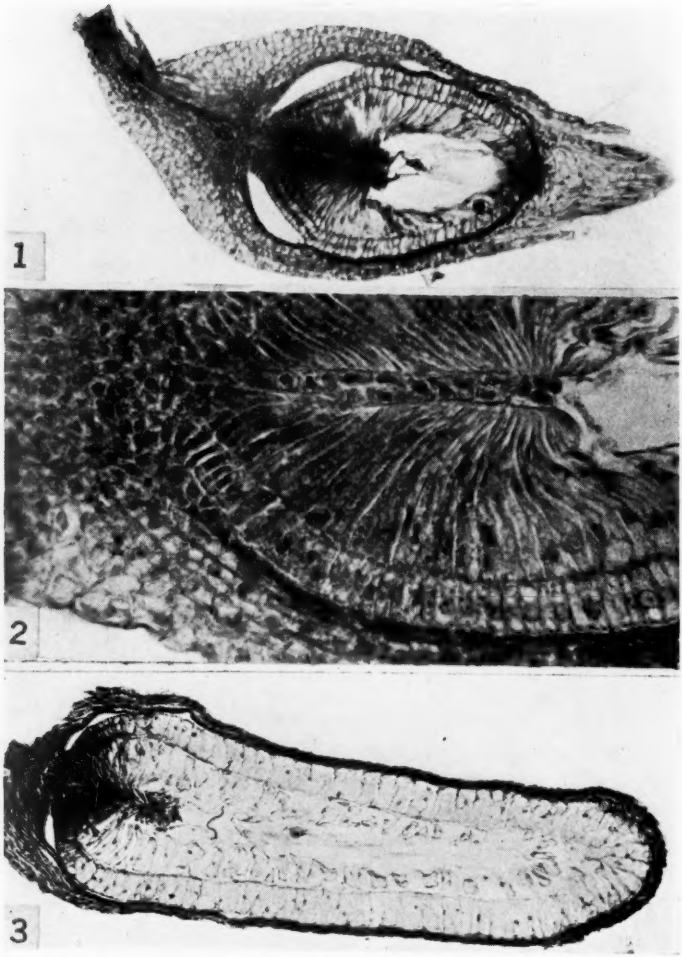
The orthotropous ovules, appearing in clusters near the top of the locule, at first grow slightly out from the placenta and then downward. By the time they are approximately twelve cells long (about 90 μ), a more or less prismatic archesporial cell appears at the apex just under the epidermis. Somewhat back of the apex, near the middle, the inner integument is initiated. No further differentiation

is to be seen in the ovule at this phase, except that an axillary row of cells just back of the archesporial cell indicates the beginning of a conductive strand.

The inner integument grows rapidly, is two layers of cells thick, and when it has about closed over the end of the nucellus the ovule is but 0.2 mm. in length. It is followed directly by the growth of the somewhat more massive outer integument. As this outer integument begins to grow up around the inner, the latter forms a pluglike growth of intertwining cells. This plug for a time projects beyond the apex of the young ovule (fig. 1). Finally, in the ripe seed the outer integument grows out beyond it in finger-like outgrowths which inclose it like a pair of paws.

Nucellus

The epidermal layer of the nucellus rather early undergoes a periclinal division, forming a two-layered tissue about the megaspores. Until the two-nucleate stage of the megagametophyte, and even later, the cells of the outer layer remain more or less cubical in shape. The cells of the inner layer become elongated in a radial direction, however, all tending to point toward the chalazal megaspore. These cells extend somewhat back of the megaspore and surround the conducting strand which traverses the chalaza, where they undergo the most extreme development. When the megagametophyte is but two-nucleate, these cells are decidedly elongated and wedge-shaped, their narrow ends being toward the center. The protoplasm with the nucleus is concentrated toward the outer, larger end of the cell, while the narrower inner end is occupied by a more or less homogeneous substance that does not readily take the haematoxylin stain. The outer portions of these cells increase considerably in size as the ovule matures. When the megagametophyte is in the two-nucleate phase, they are 14 μ in length; by the time the polar nuclei fuse they are 36 μ in length; and later they may enlarge to more than three times the size they were at the two-nucleate stage. While the outer portion of these cells becomes enlarged and remains densely protoplasmic, the inner portion is drawn out, becomes strongly tapering, and the radiate appearance is considerably accentuated (fig. 2). The walls at the narrow inner ends of the cells



FIGS. 1–3.—Fig. 1, section of ovule showing conducting strand in funiculus and outgrowth of integuments at the micropyle. Fig. 2, chalazal end of ovule showing axillary strand passing through inner nucellar tissue to antipodal end of megagametophyte. Conspicuous bricklike layer of outer nucellus matures into the perisperm. Fig. 3, ovule at time of fertilization. Note wall across megagametophyte dividing it into small basal and a large micropylar cell. Fig. 1, ×140; fig. 2, ×340; fig. 3, ×100.

become thickened and eventually no protoplasmic contents can be seen in this area.

During later development, the outer protoplasmic portion of these cells is absorbed by the enlargement of the megagametophyte. The thickened walls of their inner parts remain about the conducting strand and form the so-called postament, which projects into the older megagametophyte (fig. 3) and remains even in the mature seed, where it is imbedded in endosperm. The other cells of the inner layer of the nucellus (those toward the micropyle) increase in size and appear somewhat disarranged. In the mature seed this inner nucellar layer has been crushed except for that portion lying around the sides of the postament.

The vascular bundle of the funiculus reaches the chalazal part of the ovule, in which it ends rather abruptly (fig. 1). Beyond this point there is a region in the uppermost part of the chalaza and the very base of the nucellus in which the cells are undifferentiated and parenchymatous. This is continued upward as a narrow strand of parenchymatous cells which forms the axis of the postament, where it is two cells in thickness and probably persists by virtue of its protection within the hardened postament (fig. 2).

The epidermal layer of the nucellus develops differently from that of the inner layer just discussed. These cells gradually become prismatic and finally in the mature seed form the more or less truncated wedge-shaped cells of the perisperm. When the megagametophyte is two-nucleate these cells are for the most part cubical; at this time they are about 3.5 μ on a side. While the megagametophyte is developing to the eight-nucleate stage they enlarge considerably; particularly in the radial direction. This growth is most marked toward the apex of the ovule, where the cells become about 16 μ long by 7 μ wide. This outer nucellar layer becomes very conspicuous and appears like a wall of bricks surrounding the inner structure of the ovule. At the chalazal end this layer is interrupted by the small region of more or less undifferentiated chalazal tissue (fig. 2). During the rapid growth of the ovule following fusion of the polar nuclei, anticlinal division occurs, since the number of epidermal cells visible in a single plane doubles during this period.

When the pollen tube enters, the cells of this layer are becoming

filled with a clear, transparent substance. This development is slowest at the micropylar end, the cells there retaining their normal protoplasmic appearance until after the pollen tube has passed through the tissue.

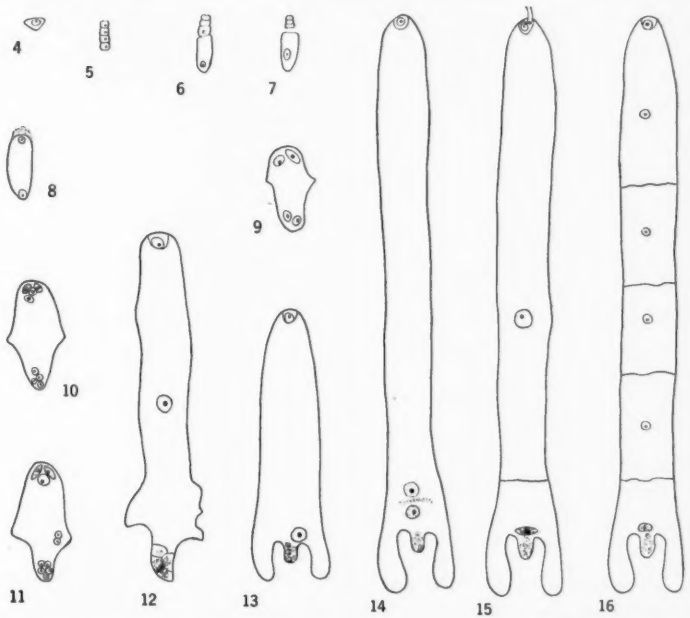
MÜCKE discussed this tissue in the mature seed of *Acorus gramineus* and concluded that the substance, which in the ripe, dry seeds is glassy, hard, homogeneous, and fills the cells completely, is cell contents and not cell wall material. There is nothing in the mature seeds to offer morphological proof of this. His conclusions were based on the chemical nature of the substance, which he proved to be protein. Sections showing the development of the perisperm confirm MÜCKE's interpretation. Soon after this substance appears in the cells, their radial walls become wavy, as are the thin membranes mentioned by MÜCKE in the mature perisperm of the seed of *A. gramineus*. Plasmolysis, which occurs very readily in the younger stages of development of the cells, shows plainly that the material in question is cell contents and not cell wall. Further, the nucleus may often be seen imbedded in cytoplasm of a cell well permeated with this substance. As the perisperm matures the nucleus disappears entirely, and the contents of the cells become uniform throughout.

From the time of fertilization, when the perisperm cells are about $57 \times 32\ \mu$ in radial section, to the mature seed, they enlarge enormously, attaining a measurement of about $230 \times 60\ \mu$ in radial section. They are roughly in the form of hexagonal prisms curved so that their inner ends all tend to point toward the chalaza.

DEVELOPMENT OF MEGAGAMETOPHYTE

A semidiagrammatic representation of the megagametophyte development is shown in figures 4–16. The archesporial cell which appears in the apex of the nucellus gives rise to an axial row of four megaspores, the chalazal one of which develops into the megagametophyte. The two-nucleate megagametophyte is about $40\ \mu$ long, and shows strong polarity, a large vacuole occupying the center. When the megagametophyte has enlarged to about $70\ \mu$ in length, it contains four nuclei. It begins its enlargement at the expense of the inner nucellar layer in the region near the micropyle, where a

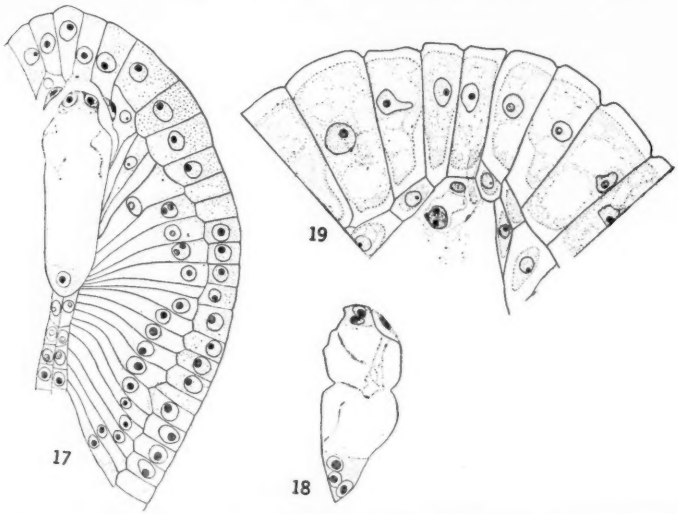
pocket-like cavity develops. Later the egg apparatus lies in this cavity. Without the megagametophyte having undergone any conspicuous enlargement, the four nuclei divide simultaneously.



FIGS. 4–16.—Fig. 4, archesporial cell. Fig. 5, tetrad of megaspores. Figs. 6, 7, degeneration of micropylar megaspores and enlargement of functional chalazal megaspore. Figs. 8–11, development of megagametophyte up to time of fusion of polar nuclei. Fig. 12, rapid elongation and enlargement of megagametophyte; synergids have disappeared. Fig. 13, postament formed and fusion nucleus moved to a point just over antipodals. Fig. 14, fusion nucleus dividing near base of megagametophyte. Fig. 15, fertilization taking place after a nucleus has been cut off in a basal cell. Fig. 16, commencement of endosperm formation. Figs. 4–12, ×250; figs. 13–15, ×100; fig. 16, ×70.

In eight-nucleate megagametophytes the two synergids are for a time very conspicuous by virtue of their dense nucleoplasm and the dense cytoplasm directly surrounding the nuclei (fig. 17). Adjacent to these are darkly staining disorganized cells of the inner nucellar layer, at whose expense the megagametophyte has increased in size.

A physiological function connected with the disorganization of adjacent cells has been ascribed to such synergids (4). That the synergids in this case perform such a function seems probable, since as soon as the few cells that are resorbed at the apex of the megagametophyte are entirely gone, the synergids themselves begin to disorganize. This degeneration of synergids is well under way when the polar nuclei fuse, and soon after this the synergids disappear en-



FIGS. 17–19.—Fig. 17, eight-nucleate megagametophyte and two layers of nucellus cells. Fig. 18, section of same eight-nucleate megagametophyte as in fig. 17. Fig. 19, apical portion of megagametophyte and adjacent nucellar layers at time of fertilization. Figs. 17, 18, ×389; fig. 19, ×439.

tirely. The egg nucleus is conspicuous and lies at the extreme micropylar end of the megagametophyte. Adjacent to it may be found for a time a polar nucleus (fig. 18). At the antipodal end are the three nuclei destined to remain as antipodals, together with the one polar contribution from that end. The polar nuclei migrate to the center or to a point near the center of the megagametophyte. This migration of the polar nuclei and their subsequent fusion is a rapid process. The megagametophyte with eight nuclei measures about 65 μ in length and shows no signs of marked increase in width.

The beginning of rapid enlargement of the megagametophyte is concurrent with the fusion of the polar nuclei. It commences laterally and toward the base at the expense of nucellar and chalazal tissue. The nucellar cells begin to collapse around the middle portion of the megagametophyte. They look very much as if they were being crushed mechanically, the pressure being localized near the center of the ovule. This may possibly be explained by the differential hardening of tissues at the time this growth begins, since this point or region where enlargement is taking place is just above a modified "hardened" area. This area consists of the attentuated inner ends of the cuneate cells of the inner nucellar layer about the antipodal end of the megagametophyte. Basal enlargement of the megagametophyte continues by the resorption of the softer parts of the nucellus around this more resistant tissue which immediately surrounds the conducting strand. This results in the peculiar formation of the postament.

After the polar nuclei have fused, a marked elongation of the micropylar half of the megagametophyte is accompanied by rapid growth of the surrounding tissues, which is due in part to increase in number of cells, but also to the rapid increase in size of the individual cells themselves. The megagametophyte triples its length before the postament itself is evident at all. In an ovule which is ready for fertilization the megagametophyte measures about 675 μ in length. Shortly after fertilization, when the endosperm has begun to form, the ovule is nearly double this size, and the embryo sac is 1340 μ long. It continues to increase, until in the mature seed the endosperm with its inclosed embryo measures about 2 mm. in length.

When the polar nuclei have fused, the conspicuous fusion nucleus remains at first near the center of the cell. Through differential growth of the megagametophyte this nucleus appears farther and farther from the center and proportionately nearer the antipodal end of the megagametophyte. Finally, before dividing, it actually does move downward so that it comes to lie adjacent to the antipodals.

By division of one or two of the antipodals the number is increased, so that in some instances there are five. In these appear a number of large, irregular, deeply staining granules. The whole

nucleus eventually stains very dark with haematoxylin, while the conspicuous dense granules are still present. Thin cell walls form about these nuclei but no further development occurs. As the megagametophyte matures they occupy a small cavity at the apex of the postament, which projects into the megagametophyte, this cavity being the constricted antipodal end of the younger megagametophyte.

After the fusion nucleus moves to the basal end of the megagametophyte, division takes place immediately. This mitosis is followed by the formation of a cell plate which divides the megagametophyte transversely into two parts, a small basal cell and a larger one comprising the main body of the megagametophyte (fig. 3). As to the general appearance of the megagametophyte at this time, both resulting cells have a relatively small amount of protoplasm in comparison with the size of the vacuole. Around the postament the cytoplasm is denser than elsewhere, and in this, just over the antipodals, lies the lenticular nucleus of the basal cell. The other daughter nucleus, that which occupies the larger, main part of the megagametophyte, moves toward the center of this cell.

FERTILIZATION

In one ovule sectioned, a flattened nucleus, obviously a sperm nucleus, appears closely appressed to a spherical nucleus which is undoubtedly the egg (fig. 19). The collapsed remains of a pollen tube which has passed through the nucellus just above the egg apparatus are present in the same section. Near the end of this pollen tube there is a small body that may represent either the tube nucleus or a second sperm. It is noteworthy that the main body of the megagametophyte is already divided into two large chambers by a cross wall. There is no indication that a second sperm is to fuse with the large nucleus which occupies the center of the larger, micropylar chamber. In none of the ovules examined is there an instance where a sperm seemed to be fusing with this nucleus, and there is no indication of a sperm moving to it in the megagametophyte under consideration. Possibly the second sperm never reaches the megagametophyte.

The possibility that a second sperm nucleus is discharged from

the pollen tube raises an interesting question: does it affect the whole endosperm or only the micropylar tissue? Obviously if the megagametophyte cavity has been previously separated into two chambers following division of the fusion nucleus, the sperm nucleus can fuse only with the daughter nucleus in the micropylar chamber. Thus endosperm in the micropylar portion might be triploid while that arising in the basal chamber would be diploid. If there is such a functioning second sperm one answer to the question lies in knowing when it enters. Here the evidence, while falling somewhat short of complete demonstration, is convincing. In ovules with megagametophytes about 300 μ long, taken from spikes in which pollination was actively occurring, there was no evidence of pollen tubes anywhere within the ovules. At this stage the fusion nucleus is lying at the base of the megagametophyte, adjacent to the antipodals.

On the other hand, by the time the megagametophyte has reached a length of 700 μ, pollen tubes are conspicuously present, some of them still unopened within the nucellus, and others that appear to have recently discharged their contents into the megagametophyte. The ovule already described as showing fertilization is in this stage. In all such ovules the fusion nucleus has already divided, a wall has been formed across the lower part of the megagametophyte, and the upper endosperm nucleus has moved far up toward the middle. While intermediate stages have not been seen, it seems wholly unlikely that in the apparently brief interval between the entry of the pollen tube and the stage just described there would be time for the second male nucleus to traverse the whole length of the very long megagametophyte, for it to unite with the fusion nucleus, and then for the latter to divide and attain the stage just described.

The cytological condition of the endosperm should throw light on these problems, but it has proved very difficult to obtain preparations of endosperm showing mitotic figures. Although ovules were killed at various hours of the day and night in an attempt to secure division stages, only one mitotic figure has been seen, and that was in telophase with the spindle oriented parallel to the plane of the section, so that it was impossible to count the chromosomes.[1]

[1] The diploid number, determined by A. Orville Dahl from sections of root tips, is 18.

ENDOSPERM

It is fairly certain, then, that the nucleus in the basal cell of the embryo sac is diploid. That the nucleus in the main cell of the embryo sac is diploid rests only on negative evidence. The tissues resulting from these two nuclei, however, are somewhat different.

In the basal cell the nucleus is rather dense, which is likewise true of the cytoplasm immediately surrounding it. This nucleus does not undergo further development immediately. The one in the larger, micropylar cell, however, by a series of divisions followed by transverse walls produces a row of cells down the center of the embryo sac. It cannot be said, in the light of the present study, whether the endosperm formation in this upper cell is initiated by free nuclear division or whether it is cellular from the start, but it is obvious that if a stage of free nuclear division occurs it is very brief. Transverse divisions continue for some time in the main part of the endosperm, so that several transverse chambers are produced. Soon, however, these are broken up into irregular cells by the formation of oblique and longitudinal walls. The endosperm ultimately is composed of uniform small cells with moderately substantial walls. It is formed rapidly and comes to occupy practically all the space inside the perisperm, enlarging at the expense of the inner nucellar cells. It is completely developed while the embryo is still young. The center of it is then digested away by the growing embryo.

Meanwhile the basal cell formed by the first division of the fusion nucleus remains unchanged for some time, at least until four cells have been formed in the upper part of the embryo sac. At length it divides repeatedly and gives rise to a more or less dense mass of irregular cells that push in around the postament and cover its upper end. For a time this tissue is conspicuously different from the rest of the endosperm, having smaller and less vacuolate cells and denser nuclei; it gradually loses its distinctive appearance as the seed matures. The original septum that cuts off this part of the embryo sac becomes difficult to distinguish in later stages of seed development, but traces of it can be noted even in the mature seed (2).

Embryo

The embryo develops somewhat more slowly than the endosperm. The two-celled embryo lies in an embryo sac in which the endosperm consists of a series of transverse cells. The first division of the zygote is transverse to the axis of the embryo sac. The upper cell then divides in a plane parallel to this. Usually each of the resulting three cells undergoes a transverse division, following which longitudinal divisions first occur. Development up to this point is not rigid, however, as the initiation of longitudinal walls may occur sooner with the formation of a shorter, stubbier proembryo. The basal cell is larger than the others and does not appear to divide more than once after it is first formed by the division of the zygote.

The embryo now rapidly becomes pyriform, all the cells but the basal one undergoing one or more longitudinal divisions, the apical cells producing most of the growth. By the time the embryo is 0.55 mm. long, differentiation is already apparent. The plumule is evident and the procambial strand can be seen in the cotyledon and extending down into the hypocotyl. The embryo continues to enlarge, utilizing the already formed endosperm as it grows up through its center. The mature embryo finally occupies the axis of the elongate endosperm and extends for about three quarters or more of its length. In the mature seed it is about 1 mm. long. At its base is a short cylindrical suspensor. Although this is frequently not obvious in longitudinal sections, it occasionally shows up clearly at the base of the mature embryo.

Summary

1. Except for the early division of the fusion nucleus followed by the formation of a cross wall dividing the megagametophyte into two large chambers before fertilization, the development of *Acorus calamus* follows the fundamental type (Normaltypus of Schnarf 7).

2. The endosperm is of the basal apparatus type. In this type the daughter nucleus, which remains at the base of the megagametophyte after the first division of the fusion nucleus, produces a tissue different in character from the other nucleus which gives rise to the main part of the endosperm. With very few exceptions the Araceae are characterized by this type of endosperm.

3. The particularly interesting feature about *Acorus calamus* is the division of the fusion nucleus before the pollen tube enters and the consequent segregation of a diploid nucleus in the basal cell before fertilization occurs.

I wish to thank Dr. F. K. BUTTERS and Dr. C. O. ROSENDAHL, who suggested the problem and gave their valuable advice during the course of this work; and Dr. R. R. HUMPHREY and Dr. E. L. NIELSEN for their aid in the collection of material. This research was done in the botanical laboratories of the University of Minnesota.

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STIMULATING EFFECT OF BETA(3)INDOLEACETIC ACID ON SYNTHESIS OF SOLID MATTER BY BEAN PLANTS[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 489

JOHN W. MITCHELL AND CHARLES L. HAMNER

(WITH FIVE FIGURES)

Introduction

Recent investigations have shown that hormones can alter the growth rate of roots and shoots (**1, 7, 8, 9, 10**), affect the morphological and histological development (**2, 3**), and to some extent control the transfer of some carbohydrate and nitrogenous compounds (**5**) within certain plants grown in darkness. In connection with these investigations, it was considered desirable to study the effect of beta(3)indoleacetic acid on the assimilation of solid matter by plants. For this purpose several thousand bean plants were grown under greenhouse conditions, and the fresh and dry weights of different portions of treated and untreated plants were compared at the beginning and end of given periods following treatment.

Investigation

MATERIALS AND TREATMENT.—Kidney beans, *Phaseolus vulgaris*, were used as experimental material. In each experiment several thousand seeds were selected for size and uniformity and planted at an even depth in quartz sand, or in the case of preliminary experiments in soil contained in 5 inch clay pots. They were watered with warm tap water and placed in a greenhouse at 75°–80° F. The relative humidity varied from approximately 40 to 80 per cent, but was generally above 60 per cent. The light intensity varied from approximately 200 foot candles, on very dark cloudy days, to approximately 5000 foot candles on clear days in early November, and 2000–3000 on clear days during December and January.

[1] This investigation was aided in part by a grant to the University of Chicago from the Rockefeller Foundation.

The plants were usually 3–4 inches above the sand level on the seventh day after planting. The sand was flushed with nutrient (**6**) and seed coats were removed so that further development was not impeded. After ten days the plants were 7–9 inches high and the second internode from 1 to 1.5 inches in length.

A number of uniform plants were then selected sufficient to allow for approximately 225 plants in each treatment, including initial and final controls. A tag, marked to correspond to the particular treatment, was placed in each pot. In this way any individual treatment was scattered through the entire population.

The 2 per cent indoleacetic acid lanolin mixtures used in some experiments were prepared by adding 100 mg. of indoleacetic acid (Merck) to 5 gm. of anhydrous lanolin contained in a small vial. The lanolin was melted and the mixture thoroughly stirred until it cooled and solidified.

Various other concentrations of indoleacetic acid were prepared by dissolving the acid in 95 per cent ethyl alcohol. The desired concentration was then prepared by adding the correct number of drops of the alcoholic solution to a weighed portion of lanolin, with the aid of a pipette. To prepare a more dilute mixture, the original alcoholic solution was diluted 1 volume to 9 with 95 per cent alcohol, and the same number of drops of the resulting solution added to another portion of lanolin of the same weight. This process was repeated until a series of concentrations was obtained. The alcohol, which remains on the surface of the lanolin, was immediately evaporated by means of a stream of warm air, and the indoleacetic acid was finally stirred into the mixture as previously mentioned.

Prior to treatment, the stems of approximately 225 plants were severed at the second internode 1.5 cm. above the point of divergence of the primary leaves. These plants were then harvested and designated as initial controls. The stems of another group were severed in like manner and 3–4 cu. mm. of pure lanolin was applied to the freshly cut surface. These were designated as final controls and harvested at the end of the experiment together with the treated plants. Stems of remaining plants were severed and 3–4 cu. mm. of the desired concentration of the lanolin mixture applied to the cut

surface. Following treatment, the pots were flushed with nutrient solution every third day and moistened with tap water on intervening days.

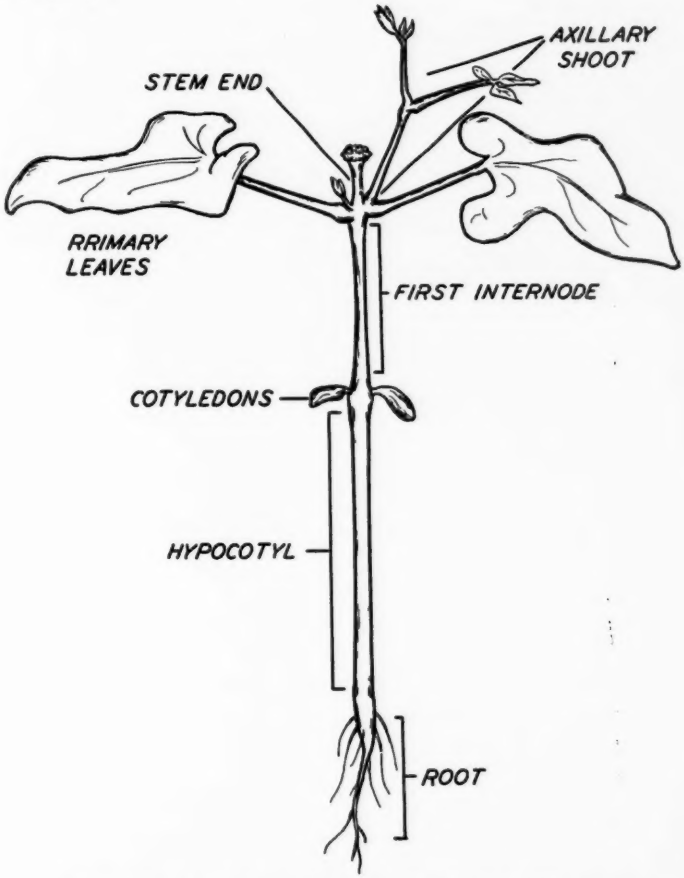

FIG. 1.—Parts into which plants were divided for weight determinations

In some experiments it was desirable to prevent the growth of new shoots which developed in the axils of the leaves soon after the stems were severed. The newly developed axillary buds, 1–3 mm.

in length, were therefore carefully removed and discarded at intervals during the experiments.

DETERMINATION OF FRESH AND DRY WEIGHTS.—At harvest the roots were first washed free from most of the sand, then dipped into a saturated solution of sodium chloride for approximately 30 seconds to loosen the remaining sand. The roots were immediately washed and the plants carefully divided into the following fractions: (1) roots, (2) hypocotyls, (3) cotyledons, (4) first internodes, (5) primary leaves and petioles, (6) axillary shoots together with leaves and petioles, and (7) the treated portion of the second internode later designated as stem end (fig. 1).

The fresh weight of each fraction representing the parts of approximately 225 plants was recorded. The fractions were chopped fine and placed in a well ventilated drying oven at 80° C. for a period of 24–30 hours. The dry weight of each fraction was then determined by means of a torsion balance which was sensitive to 0.05 gm., or in the case of small samples, by means of an analytical balance.

The leaf areas of some of the plants were measured by means of a photoelectric method (**4**).

In all experiments the results were obtained from samples representing approximately 225 plants. Previous experiments (**5**) with plants grown in the dark and also preliminary experiments with plants grown under greenhouse conditions showed that duplicate samples from this number of plants did not differ more than 3 per cent in dry weight. As a matter of convenience the data are presented on the basis of 100 plants.

Results and discussion

MORPHOLOGICAL OBSERVATIONS.—The first apparent response of plants to concentrated mixtures (0.2–2.0 per cent) of indoleacetic acid occurred within 48 hours after treatment. The stems changed from dark to light green for a distance of 3–5 mm. below the point of application. Tumors and roots, similar to those observed in previous experiments (**3**), later developed directly below the treated surface. When more dilute concentrations were used (0.002 per cent or less), there was less change in the color of the stem ends and no

noticeable enlargement of the stem below the point of treatment. Transverse sections through this region of plants treated with 0.000185 and 0.0000185 per cent of indoleacetic acid showed that roots were not initiated during a period of six days. However, plants treated with the most dilute concentrations formed more callous tissue at the cut surface than did control plants treated in a similar manner with pure lanolin. The amount of proliferation of tissues at the stem end varied with the lighting conditions, as relatively small tumors were produced by treated plants during dark cloudy days of December and January.

TABLE 1

DRY WEIGHT GAINED BY PARTS OF UNTREATED PLANTS AS COMPARED WITH THOSE OF PLANTS TREATED WITH 2 PER CENT INDOLEACETIC ACID. FIGURES REPRESENT GRAMS SOLID MATTER GAINED PER 100 PLANTS DURING PERIODS OF FIVE DAYS (EXPERIMENT 1) AND SIX DAYS (EXPERIMENT 2)

PARTS	CONTROL	TREATED
Experiment 1		
Leaves	9.6	9.7
Axillary shoots	12.6	5.7
Stem ends	0.68	2.89
Experiment 2		
Leaves	14.7	15.8
Axillary shoots	17.6	8.5
Stem ends	1.06	4.15

Preliminary experiments showed that plants treated with concentrations greater than 0.002 per cent developed smaller axillary shoots than did control plants. No noticeable difference, however, was observed between the growth of axillary shoots of control plants as compared with those of other plants treated with concentrations less than 0.002 per cent. There was no apparent difference in the size and color of the primary leaves of treated and untreated plants.

FRESH AND DRY WEIGHTS.—As initial experiments, plants grown in soil were treated with 2 per cent indoleacetic acid lanolin mixture. Control plants, together with an equal number of treated plants, were harvested at the end of five or six days following treatment.

The dry weights of primary leaves, stem ends, and axillary shoots were determined. The results show that indoleacetic acid caused solid matter to move toward the point of application, and that development of the axillary shoots was definitely retarded owing to its application (table 1).

More extensive experiments were then conducted, first to study the effect of indoleacetic acid on the dry weight of different parts of the entire plant, during an extended period of treatment, and

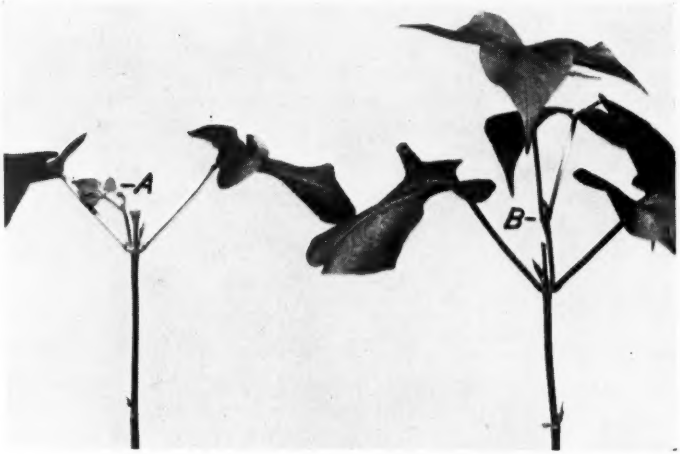

FIG. 2.—Growth of axillary shoots of untreated plant (*B*) compared with that of plant treated with 2 per cent mixture of indoleacetic acid and lanolin (*A*). Picture taken eight days after treatment.

second to determine its effect on the total amount of solid matter synthesized by plants during a given period.

For this purpose plants grown in sand were treated with 2 per cent indoleacetic acid lanolin mixture. Controls, together with an equal number of treated plants, were harvested at frequent intervals during the following period of fifteen days.

During the first three days following treatment one or two dormant buds in the axils of the primary leaves of control plants became active and grew rapidly, while on the other hand the stem end of treated plants showed active growth and the axillary buds re-

mained dormant. Thus during this period the axillary buds of control plants gained approximately 1.7 gm. of solid matter and the stem ends of similar plants gained only 0.2 gm. per 100 plants. In contrast to this, the axillary shoots of treated plants gained only 0.6 gm. and the stem ends of treated plants gained 1.1 gm. of solid matter per 100 plants (fig. 2, table 2).

From the third to the sixth day following treatment the axillary shoots of controls grew rapidly, withdrawing practically all neces-

TABLE 2

COMPARISON OF GAIN IN DRY WEIGHT OF PLANTS TREATED WITH 2 PER CENT INDOLEACETIC ACID WITH THAT OF UNTREATED PLANTS. FIGURES REPRESENT GRAMS DRY WEIGHT PER 100 PLANTS

Parts	3 days		6 days		9 days		15 days	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Stem ends	0.203	1.143	0.447	2.752	0.689	4.259	1.009	5.179
Axillary shoots	1.691	0.670	10.7	3.76	29.8	13.1	60.6	26.9
Primary leaves	9.90	7.48	5.82	9.82	12.75	13.72	5.94	7.17
First internodes	0.88	1.02	1.39	2.25	1.88	2.80	1.94	2.91
Hypocotyls	1.69	2.10	1.24	2.14	1.39	2.95	3.98	2.76
Roots	4.34	3.96	3.55	3.91	5.43	5.23	7.20	6.65
Total	18.70	16.38	23.15	24.63	51.93	42.06	80.67	51.57
Total minus axillary shoots	17.01	15.71	12.45	20.87	22.13	28.96	20.07	24.67

sary nutrients from the other parts of the plants, as the leaves of the young shoots were not yet expanded. This is shown by the fact that the axillary shoots of 100 control plants gained approximately 9 gm. of solid matter while the remainder of the same plants lost 4.5 gm. during this period. The leaves of the young shoots of control plants expanded and became active photosynthetically soon after the sixth day following treatment, and all parts of the plants showed a gain in solid matter following that date. In contrast to this, the growth of axillary shoots on treated plants was inhibited and solid matter moved largely toward the point of treatment instead of into the axillary shoots.

During the six days following treatment, both treated and control

plants were largely dependent upon their primary leaves (which were of approximately the same area) as a source of carbohydrates. The effect of indoleacetic acid on the total amount of solid matter synthesized by treated and untreated plants during this time can therefore be compared on the basis of approximately equal leaf areas. Such a comparison shows that during the first three days, indoleacetic acid slightly retarded the synthesis of solid matter, but from the third to the sixth day 100 treated plants gained approximately 8.3 gm. of solid matter while the same number of untreated plants gained only 4.5 gm. Thus indoleacetic acid not only causes an accumulation of solid matter near the point of application, but it also stimulates the synthesis of solid matter by the plant. This stimulation in rate of synthesis of solid matter was not evident after approximately the sixth day, however, as the control plants developed new axillary shoots and leaf surface more rapidly than did treated plants.

In general the results show: (1) that extension of the stem axis and development of new leaves was retarded by the treatment (fig. 2, table 2); (2) that those parts of treated plants nearest the point of application (stem ends, primary leaves, and first internodes) gained more solid matter than did similar parts of control plants; and (3) that the total gain in solid matter by treated plants was at first slightly less than that of controls, but between the third and sixth day the treated plants gained approximately twice as much solid matter as did the controls. After about the sixth day the control plants accumulated weight more rapidly, probably because of their greater leaf area than that of the treated plants. Variations in fresh weights of treated and untreated plants were similar to those observed in the case of the dry weights.

Further experiments were conducted to study in detail the effect of different concentrations of indoleacetic acid on the rate at which plants synthesized solid matter. The factor of bud inhibition was eliminated in order to simplify the experiment, and to determine definitely whether indoleacetic acid affected the amount of solid matter synthesized, irrespective of its effect on bud development.

Plants were treated with various concentrations of indoleacetic

acid, newly developed axillary buds removed from both treated and control plants, and weight determinations made at the end of the following six day period. The experiment was repeated four times during the months of December and January.

On the basis of 100 plants, those grown during the interval of December 4–10 and treated with 0.000185 per cent indoleacetic acid

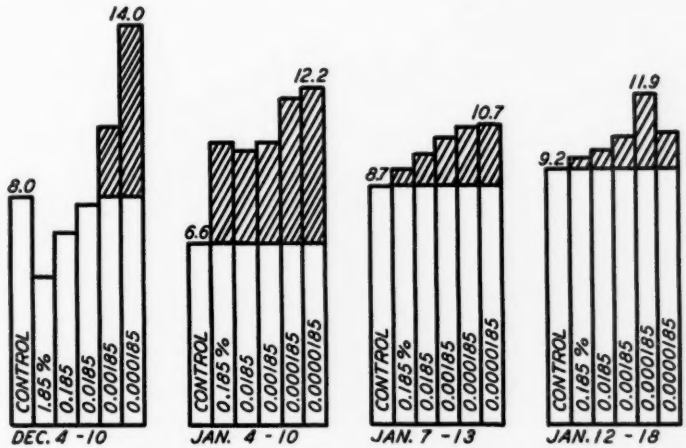

FIG. 3.—Gain in dry weight by four sets of plants, each group treated with indoleacetic acid lanolin mixtures covering similar range of concentrations. Plants of each group were of slightly different ages at time of treatment. Axillary shoots removed, leaving primary leaves as photosynthetic organs. Each column represents grams dry matter synthesized by 100 plants during six days. Shaded portions represent dry matter synthesized by treated plants in excess of that gained by controls.

gained 14.0 gm. of dry matter, while the same number of untreated plants gained only 8.0 gm. Similarly 100 plants grown between the fourth and tenth of January and treated with 0.0000185 per cent indoleacetic acid gained 12.2 gm. of solid matter, whereas a like number of untreated plants gained 6.6 gm. (fig. 3, table 3). The fresh weight of treated and untreated plants varied in approximately the same way. During these two experiments the average light intensity was relatively high. Thus the plants were stimulated in their ability to synthesize solid matter, when treated with a low concen-

tration of indoleacetic acid and grown during a period of relatively high light intensity. Treated plants of other groups grown during dark cloudy weather were much less affected (table 4).

Although the plants used for any single experiment were similar in size, appearance, and age, they varied between experiments at the

TABLE 3

RESULTS OF TREATING WITH DIFFERENT CONCENTRATIONS OF INDOLEACETIC ACID. FIGURES REPRESENT GRAMS DRY MATTER GAINED BY 100 PLANTS IN EACH OF FOUR EXPERIMENTS, DURING A PERIOD OF SIX DAYS

DATE OF EXPERIMENT	CONTROL	PERCENTAGE CONCENTRATION					
		1.85	0.185	0.0185	0.00185	0.000185	0.0000185
December 4–10..	8.03	5.32	6.99	7.99	10.74	14.02	.....
January 4–10....	6.62	.....	10.34	9.81	10.14	11.66	12.20
January 7–13....	8.67	.....	9.19	9.82	10.33	10.67	10.69
January 12–18...	9.19	.....	9.60	9.98	10.44	11.94	10.54

TABLE 4

EFFECT OF LIGHT INTENSITY ON RESPONSE OF PLANTS TO DILUTE CONCENTRATIONS OF INDOLEACETIC ACID. FIGURES REPRESENT MAXIMUM INCREASE IN GRAMS OF DRY WEIGHT PER 100 PLANTS OVER A PERIOD OF SIX DAYS

DATE OF EXPERIMENT	HOURS OF SUNSHINE	GAIN OVER THAT OF CONTROLS
December 4–10.....	32.5	6.0
January 4–10.....	24.6	5.6
January 7–13.....	17.6	2.0
January 12–18.....	17.1	2.8

time of treatment because of differences in light conditions. Thus plants of some experiments were relatively sturdy while others grown during periods of dark cloudy weather were etiolated.

Both the treated and the untreated plants were dependent mainly on the primary leaves for synthesis of carbohydrates, as new buds were removed when less than 3 mm. in length. Area measurements were made to study the effect of indoleacetic acid on the rate of

expansion of these primary leaves. Table 5 shows that 0.000185 and 0 0000185 per cent mixtures caused a slight stimulation in leaf expansion by plants grown during a period of relatively high light intensity. On the other hand, the leaf expansion of treated plants was inhibited by indoleacetic acid during periods of dark cloudy weather. It is evident that there is a relationship between light intensity and effect of indoleacetic acid on leaf expansion, but more detailed experiments are necessary to establish the nature of this relationship.

TABLE 5

EFFECT OF DIFFERENT CONCENTRATIONS OF INDOLEACETIC ACID ON EXPANSION OF LEAVES. FIGURES REPRESENT SQUARE CENTIMETERS OF LEAF SURFACE PER 100 PLANTS. MEASUREMENTS MADE ON SIXTH DAY FOLLOWING TREATMENT

DATE OF EXPERIMENT	CONTROL	PERCENTAGE CONCENTRATION				
		0.185	0.0185	0.00185	0.000185	0.0000185
January 4–10.....	11460	11030	10950	11570	12120	12140
January 7–13.....	13060	11360	11620	11580	11800	11800
January 12–18.....	13750	12870	12550	12850	12900	12100

It is of importance to note that indoleacetic acid stimulated the plants to synthesize more solid matter, without causing a corresponding increase in leaf area.

It is evident from figure 4 and the data presented in table 6 that the amount of proliferation of stem tissue and the accumulation of solid matter near the point of application were proportional to the concentration of indoleacetic acid used. Thus the stem ends of 100 plants treated with 0.185 per cent mixture gained 1.36 gm. of solid matter during the interval from January 12 to 18, while those of the same number of plants treated with 0.0000185 per cent gained only 0.48 gm. during the same time. Although plants treated with concentrated mixtures showed a marked response near the point of treatment, this localized response was accompanied by a relatively small gain in the dry weight throughout the rest of the plant (fig. 5). Plants treated with more dilute concentrations showed practically

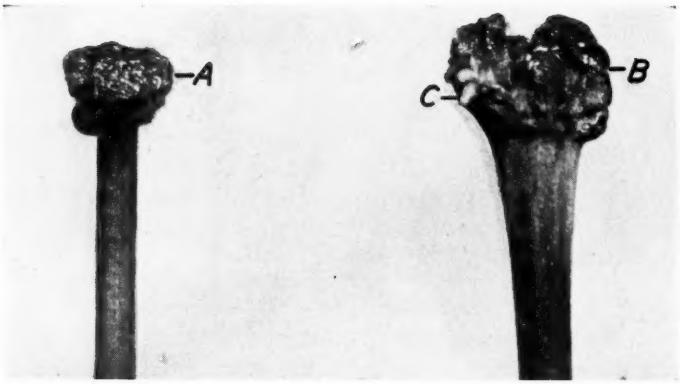

FIG. 4.—Tumor formation and root development by plant treated with 2 per cent mixture of indoleacetic acid and lanolin (*B*, *C*) as compared with callus formed by plant treated with a 0.0000185 per cent mixture (*A*). Picture taken six days after treatment.

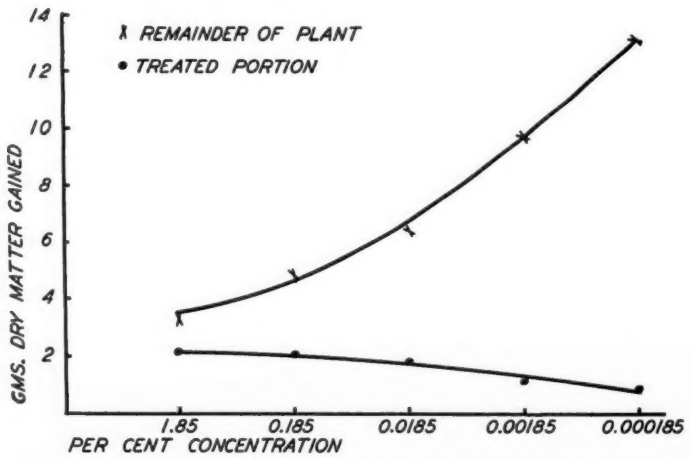

FIG. 5.—Decrease in concentration of indoleacetic acid lanolin mixtures from approximately 2 to 0.0002 per cent caused decrease in dry weight of tumors produced and marked increase in dry weight of remaining parts of plants. Figures represent grams dry matter gained per 100 plants during six days.

no response near the point of treatment, but gained more in fresh and dry weight than did plants grown under the same conditions but treated with concentrated mixtures of the acid.

In general it is concluded that indoleacetic acid is not only capable of inhibiting development of dormant axillary buds and causing the tissues of some plants to proliferate and form roots at the point of

TABLE 6

INCREASE IN DRY WEIGHT OF STEM ENDS OF PLANTS TREATED WITH VARIOUS CONCENTRATIONS OF INDOLEACETIC ACID. FIGURES REPRESENT GRAMS GAINED PER 100 PLANTS DURING A PERIOD OF SIX DAYS

EXPERIMENT	CONTROL	PERCENTAGE CONCENTRATION					
		1.85	0.185	0.0185	0.00185	0.000185	0.0000185
Dry weight							
December 4–10....	0.80	2.29	2.26	1.76	1.14	0.99	.....
January 4–10.....	0.39	.....	1.28	1.19	0.87	0.54	0.46
January 7–13.....	0.35	.....	1.57	1.49	1.08	0.47	0.56
January 12–18.....	0.48	.....	1.36	1.24	0.86	0.55	0.48
Fresh weight							
December 4–10....	7.7	17.9	18.1	15.2	11.9	12.7	.....
January 4–10.....	3.0	.....	9.0	8.2	6.0	4.5	3.6
January 7–13.....	3.0	.....	12.5	12.4	8.8	5.0	5.1
January 12–18.....	3.8	.....	8.9	8.5	6.2	5.2	4.4

application, but, particularly when used in small amounts, it is capable of stimulating the plants to synthesize a greater amount of solid matter.

Summary

1. The stem tips of bean plants were removed and lanolin containing different concentrations of indoleacetic acid applied to the cut surface. When concentrations stronger than approximately 0.00185 per cent were used, the development of axillary buds was retarded, the stem ends produced tumors and roots, and those parts of the plants near the point of treatment increased more in fresh and dry weight than did similar parts of untreated plants.

2. When concentrations of indoleacetic acid lower than approximately 0.00185 per cent were used, its inhibitive effect on develop-

ment of axillary buds was less, the plants produced much smaller tumors, and no roots were developed.

3. By removing the axillary buds from both treated and untreated plants it was possible to study the effects of indoleacetic acid other than its inhibitive action on bud development. Dilute concentrations caused plants with the axillary buds removed to gain 23–85 per cent more solid matter as compared with untreated plants during a period of six days. The final dry weights of plants treated with these concentrations were between 6 and 11 per cent greater than the final weights of untreated plants at the end of a six day period, although the average weight of all of the plants was the same at the time of treatment.

4. Plants with axillary buds removed were stimulated to synthesize a much greater amount of solid matter when grown in light of a relatively high intensity than when treated with the same concentration of indoleacetic acid and grown during a period of relatively low light intensity.

5. The area of leaf surface of treated plants with axillary buds removed was not appreciably greater than that of untreated plants at the end of a six day period of treatment.

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CYTOLOGICAL INVESTIGATIONS OF PISUM SATIVUM

GEORGE OLDS COOPER[1]

(WITH TWENTY-EIGHT FIGURES)

Introduction

Little, if any, detailed study has been made of either microsporogenesis or megasporogenesis in *Pisum sativum* Linn. HÄKANSSON (6, 7), PELLEW and SANSOME (9), RICHARDSON (10), and SANSOME (12, 13) were particularly interested in the peculiar types of chromosome associations found at diakinesis and on the heterotypic equatorial plates of certain partially sterile plants. CANNON (1) described the behavior of chromosomes in pea hybrids. Investigations in the *Vicia* tribe of the Leguminosae have been concerned chiefly with the embryo development of various species of *Lathyrus*. LATTER (8) made a study of microsporogenesis for *L. odoratus*. FISK (5) determined the haploid chromosome number to be seven for a number of species of the same genus. ROY (11) reported the development of the megagametophytes of several members of the Leguminosae, including *P. sativum*. His investigation of this species indicates that the archesporial cell functions directly as the megaspore mother cell and does not cut off the parietal cell.

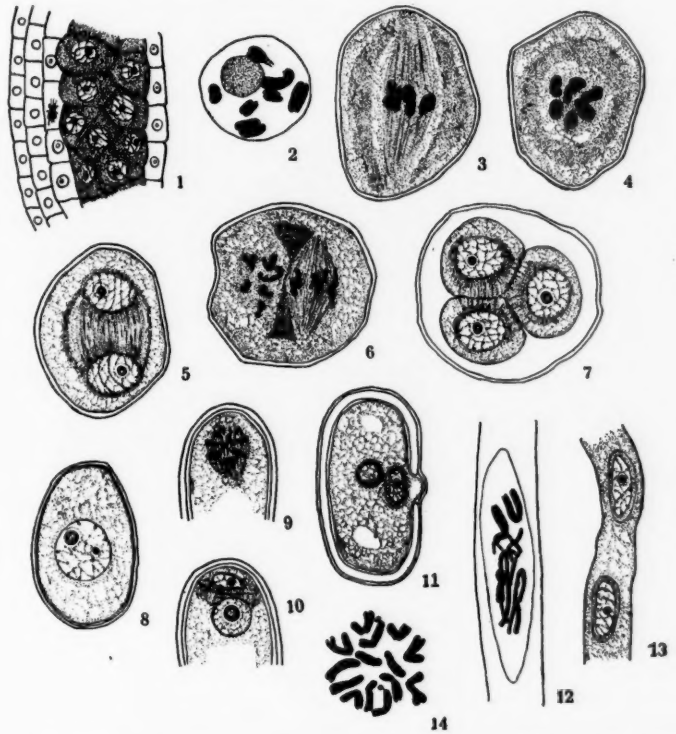
MATERIAL AND METHODS.—The material used in this investigation was from two horticultural strains of the garden pea (Little Marvel and Asgrow's Pride). Buds of various ages were fixed either in Karpechenko's modification of Navashin's solution or in Carnoy's solution, imbedded in paraffin, sectioned at 12 μ, and stained in Delafield's haematoxylin.

Investigation

MICROSPOROGENESIS.—Each theca of the anther contains many microspore mother cells, which are readily identified because of their

[1] The writer wishes to express his appreciation to the Departments of Genetics and Botany, University of Wisconsin, for the facilities made available during the course of this investigation.

size and staining qualities (fig. 1). The nuclei of the tapetal cells which surround the sporogenous tissue divide during the interval



FIGS. 1–14.—Microsporogenesis (all ×1000 unless otherwise indicated): Fig. 1, longitudinal section through anther showing sporogenous tissue; ×550. Fig. 2, diakinesis, seven pairs of chromosomes; ×1250. Fig. 3, first meiotic metaphase. Fig. 4, polar view. Fig. 5, interkinesis. Fig. 6, second meiotic metaphase; spindles at right angles. Fig. 7, cytokinesis; partition walls appearing. Fig. 8, mature microspore. Figs. 9, 10, divisions of microspore nucleus. Fig. 11, young pollen grain. Fig. 12, late prophase and division of generative nucleus; ×1450. Fig. 13, two male gametes in pollen tube; ×1450. Fig. 14, polar view, somatic division, fourteen chromosomes; ×1250.

in which those of the sporogenous cells are undergoing the prophase stages of meiosis. Each tapetal cell ultimately becomes binucleate. LATTER records the same behavior for *Lathyrus odoratus*. A marked

enlargement of the microspore mother cell nucleus occurs during the late prophase stage. Seven pairs of chromosomes are present at diakinesis (fig. 2). The chromosomes take a dense stain at this stage whereas the nucleole stains faintly. Polar views of somatic equatorial plates in root tip cells show fourteen chromosomes. Two of the chromosomes possess prominent satellites. The chromosomes are slender and variable in length, bent at the points of spindle attachment constrictions, and show arms of unequal length (fig. 14).

Immediately following diakinesis the nucleus shrinks somewhat in size, the nucleole disappears, the nuclear membrane disintegrates, and the chromosomes are massed near the center of the nuclear cavity. A multipolar spindle, which ultimately becomes bipolar diarch, forms with the chromosomes arranged at the equator midway between the poles. The paired nature of the chromosomes is readily distinguishable at metaphase (fig. 3). The seven bivalent chromosomes are easily counted in polar views of the first meiotic metaphase (fig. 4). The cytoplasmic sheath observed by COOPER (2) and COOPER (3, 4) was present. The newly formed nuclei at interkinesis do not fully take on the characteristics of a resting nucleus, but remain temporarily in a late prophase condition (fig. 5). The spindles of the second meiotic metaphase are usually at right angles to each other, a dense zone of cytoplasm from the first meiotic division separating the two spindles (fig. 6). LATTER did not describe the presence of this dense perinuclear zone in *Lathyrus*.

Subsequently a cell containing four nuclei is formed as a result of the second meiotic division (fig. 7). The spindles of this division persist for a time and cell plates are formed midway between the nuclei of the tetrad. The cell plate splits and ultimately four spores are formed, each containing one nucleus.

The spores are released from the microspore mother cell wall shortly after their formation, and are free in the theca. The microspore becomes ovoid, increases in size, and a smooth exine is developed (fig. 8). The nucleus of the mature microspore takes a position at the end of the cell and divides mitotically. The spindle lies lengthwise in the cell (fig. 9). A cell plate is laid down across the spindle and the microspore is divided to form a large tube cell and a smaller generative cell (fig. 10). The tube nucleus disintegrates

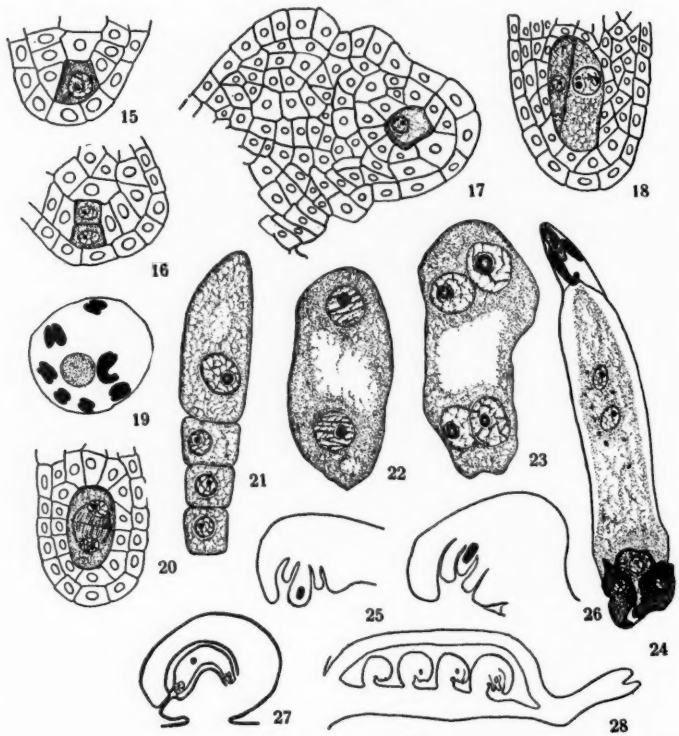
early and at the time of pollination has practically disappeared. During the course of development of the pollen grain the intine has increased in thickness, forming a heavy wall except over the germ pores. Each pollen grain has two laterally placed germ pores. A large vacuole is found within each pore, covered with a thin intine. As a rule the generative nucleus does not divide until after growth of the pollen tube. The newly formed generative cell when young is slightly ovoid in shape, but on maturation of the microgametophyte becomes linear to sickle-shaped (fig. 11).

The pollen grains are shed before the opening of the flower. The chances of cross pollination are very slight, therefore, because when the flower opens the pollen tubes have progressed far enough down the style so that foreign pollen would be ineffectual. The generative cell divides mitotically while the pollen tube is growing down the style (fig. 12). Following this division the two male gametes are surrounded by a narrow ring of cytoplasm, a thin membrane separating them from the tube cytoplasm. The gametes are carried in the cytoplasm of the pollen tube and progress with it to the micropyle of the ovule (fig. 13).

OVULE DEVELOPMENT.—The flower contains a single ovary bearing typically ten ovules borne alternately on the two placentae. All of these may not be fertilized, or if so may not continue development, so that only rarely does one find ten ripened ovules in the mature fruit. When the megaspore mother cell is at the early prophase of the first meiotic division, two rounded outgrowths appear from the placental tissue. These outgrowths develop more rapidly on one side of the ovule than on the other and the ovule bends toward the stylar end of the ovary. This bending continues as growth progresses, so that the ovule is half anatropous at maturity (figs. 25–28).

MEGASPOROGENESIS.—A hypodermal cell at the apex of the ovule becomes differentiated as the archesporial cell (fig. 15). This cell divides to form a primary parietal cell and a primary sporogenous cell (fig. 16). ROY did not observe such a division in *Pisum sativum* and regards it as an exception for this group. The primary sporogenous cell (fig. 17), now the megaspore mother cell, enlarges during the stages leading to diakinesis, and the cytoplasm is finely vacuo-

late. It was noted in several instances that there may be two or even three sporogenous cells within an ovule (fig. 18).



FIGS. 15–28.—Megasporogenesis: Fig. 15, archesporial cell; ×425. Fig. 16, primary parietal and primary sporogenous cell; ×425. Fig. 17, young ovule with sporogenous cell showing early stage in development of integuments; ×425. Fig. 18, portion of nucellus showing two primary sporogenous cells; ×425. Fig. 19, diakinesis; seven pairs of chromosomes; ×1250. Fig. 20, interkinesis; ×425. Fig. 21, linear row of spores; ×1000. Figs. 22–24, stages in development of megagametophyte: fig. 22, two-nucleate stage; fig. 23, four-nucleate stage; fig. 24, mature megagametophyte; ×825, 825, 450. Figs. 25–27, development of integuments and formation of half anatropous ovule; ×85, 85, 45. Fig. 28, longitudinal section through young ovary showing position of ovules; ×5.

Seven pairs of chromosomes are present at diakinesis (fig. 19). Two cells are formed following the first meiotic division (fig. 20).

Both nuclei divide during the second meiotic division to form two cells each. The result of these divisions produces usually four megaspores (fig. 21). The chalazal cell which becomes the functional megaspore enlarges greatly and becomes elongated.

The megaspore nucleus divides mitotically to form a two-nucleate megagametophyte. A large vacuole forms between the two nuclei, which are found at each end of the megaspore (fig. 22). Further divisions of these nuclei lead to development of a four (fig. 23) and later of an eight-nucleate megagametophyte. Three of these nuclei remain at the chalazal end of the megagametophyte, develop cell walls, and become irregularly ovoid antipodal cells. Three nuclei remain at the micropylar end and after cell wall formation become differentiated into the egg cell and the two synergids. The egg is pear-shaped and lies with the smaller end toward the micropyle. The egg may be recognized by the nature of the cytoplasm, which is finely vacuolate at the basal region of the cell and coarsely vacuolate at its tip end. The large nucleus lies in the denser cytoplasm at the base of the cell. The synergids are irregular in shape and the cytoplasm is very dense and finely vacuolate. The small, almost abortive nuclei lie in the mid regions of the synergids. The two remaining nuclei, which become the polar nuclei, remain within the old megagametophyte wall and do not unite until the time of fertilization. The mature eight-nucleate, seven-celled, elongated and curved megagametophyte consists of three small uninucleate ovoid antipodals imbedded in the nucellus, a large binucleate primary endosperm cell, two irregularly shaped synergids, and a pear-shaped egg cell (fig. 24). It was observed occasionally that two megagametophytes were present in an ovule, one larger than the other.

Summary

1. Each theca of the anther of *Pisum sativum* contains many microspore mother cells.

2. Cell plates are formed on the cytoplasmic strands midway between the nuclei of the tetrad.

3. The microspore cell divides to form a two-celled pollen grain.

4. The tube nucleus disintegrates before germination of the pollen grain.

5. The generative cell divides within the pollen tube to form two male gamete cells.

6. Each ovule usually contains one primary sporogenous cell; two or three cells have been observed in some instances.

7. An apical hypodermal archesporial cell divides to form a primary sporogenous cell and a primary parietal cell.

8. The primary sporogenous cells function as megaspore mother cells.

9. Usually four megaspores are formed as a result of the meiotic divisions.

10. The chalazal megaspore develops into an eight-nucleate, seven-celled megagametophyte; the other megaspores disintegrate.

11. One megagametophyte is usually found in an ovule; occasionally two are present.

12. The mature ovule is half anatropous.

13. Two integuments are present.

14. The haploid chromosome number is seven.

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MACROSPOROGENESIS AND EMBRYO DEVELOPMENT IN ULMUS FULVA

RUTH I. WALKER

(WITH TWENTY-EIGHT FIGURES)

Introduction

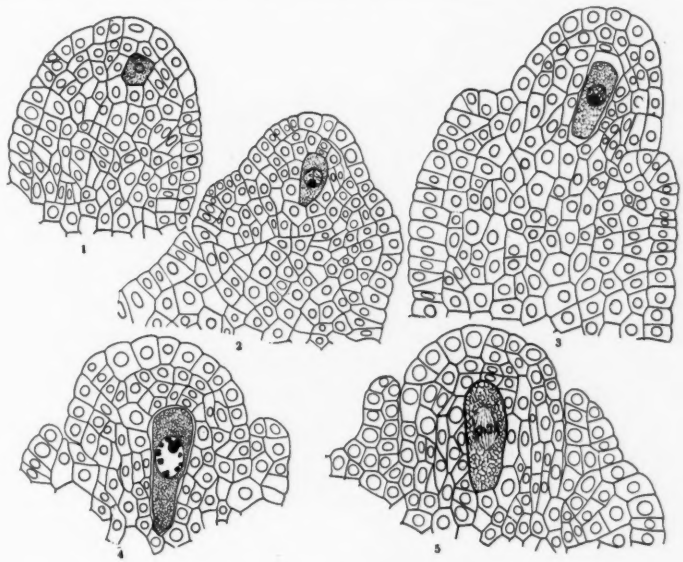
SCHNARF (**3, 5**) has summarized the various types of development of the megagametophyte in the angiosperms. In the *Adoxa* type, until recently referred to as the *Lilium* type, the macrospore mother cell develops directly into the megagametophyte, without the formation of a linear row of macrospores. SHATTUCK (**6**) has reported that this type occurs in the majority of cases in *Ulmus americana*. STENAR (**7**) described a modification of the *Adoxa* type in *Gagea lutea*, in which the macrospore mother cell undergoes two divisions, forming a linear row of four macrospore nuclei. All four nuclei may divide again, forming a typical eight-nucleate megagametophyte; or only the three nuclei nearest the micropyle may divide and the chalazal nucleus degenerates. DAHLGREN (**1**) has reported that all four macrospore nuclei enter into the development of the megagametophyte of *Armeria* and *Statice*. A similar condition in its formation has been found by HAUPT (**2**) in *Plumbago capensis* (in disagreement with DAHLGREN), but variations occur in the number and the position of the antipodals and in the number of nuclei forming the endosperm.

Material and methods

Flower buds of *Ulmus fulva* Michx. were collected during the spring of 1934, and in the spring, summer, and autumn of 1936 from a native tree growing in the suburbs of Milwaukee. This material was fixed in Karpechenko's modification of Navashin's, Flemming's medium, and Carnoy's acetic-alcohol-chloroform solutions. Since the buds of *Ulmus fulva* are very hairy and float on the surface of aqueous fixatives, they were first submerged in Carnoy's solution for a few seconds before being transferred to the Flemming's medium or Karpechenko's mixtures. The fixed material was imbedded in paraffin. Sections were cut from 8 to 20 μ in thickness and were

stained with safranin and crystal violet or with Heidenhain's iron-alum haematoxylin. A counter stain of fast green was used with the latter.

All drawings were made with a camera lucida at table level. Spencer compensating oculars, and Spencer 16 mm. N.A. 0.25, and 4 mm. N.A. 0.66 achromatic objectives and 1.5 mm. 1.25 N.A. achromatic oil immersion objective were used.



FIGS. 1–5.—Development of ovule: Fig. 1, nucellus of young ovule showing hypodermal archesporial cell. Fig. 2, ovule as it appears in the autumn showing origin of inner integument. Fig. 3, ovule the following spring; macrospore mother cell in early prophase. Fig. 4, macrospore mother cell, diakinesis. Fig. 5, heterotypic metaphase ×380.

Observations

OVULE DEVELOPMENT

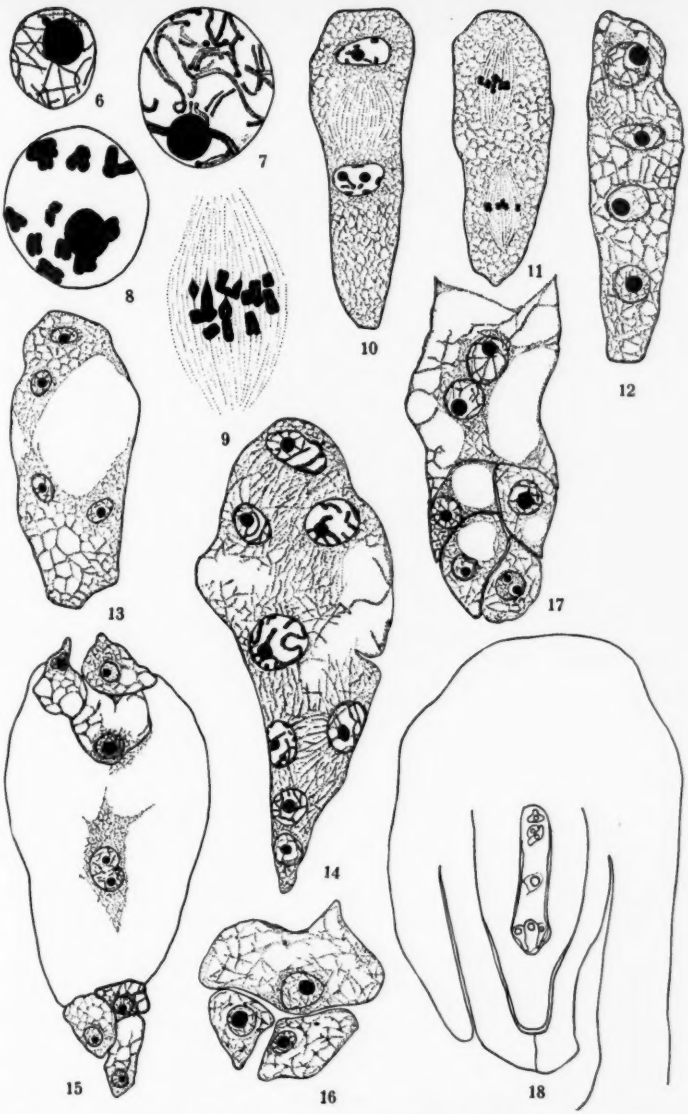
A single anatropous ovule develops in each ovary. The nucellus arises as a meristematic mass of cells on the inner surface of the ovary wall (fig. 1). The young ovule bends toward the stylar end of the ovary. The inner integument begins its development in the

autumn as an outgrowth from the epidermal layer, at a level just below that of the primary sporogenous cell (fig. 2), while the outer integument begins development the following spring, about the time of the heterotypic division in the macrospore mother cell (fig. 5). This integument arises just below the inner integument. Both integuments continue to grow and reach a level almost even with the apex of the nucellus by the time the four macrospore nuclei are formed. When the macrogametophyte is mature, the integuments inclose the nucellus, leaving only the micropyle (fig. 18).

MACROGAMETOPHYTE DEVELOPMENT

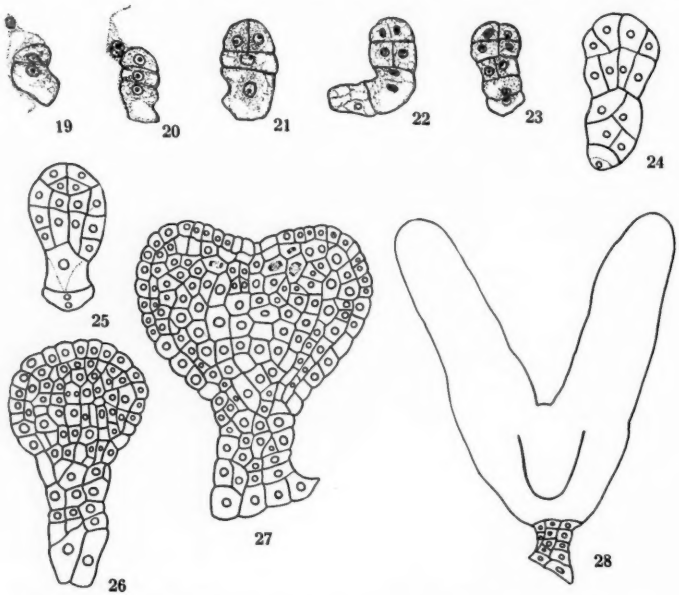
Early in August, a single hypodermal cell (fig. 1) is differentiated at the apex of the nucellus as an archesporial cell. This cell functions as the macrospore mother cell and is easily recognized by its greater size and deeper staining properties. It grows considerably in length (fig. 2). When it is about three times as long as broad, growth ceases until the following spring (fig. 3).

The nucleus of the macrospore mother cell contains a single large nucleolus, imbedded in the chromatic network (fig. 6). The latter condenses to form dense threads (fig. 7) which shorten, thicken, and appear as pairs of chromosomes (fig. 8). Figure 9 shows fifteen pairs of these at the equatorial plate of heterotypic division. The two daughter nuclei (fig. 10) resulting from the heterotypic division shortly undergo the homoeotypic division (fig. 11), and four nuclei appear in a linear row (fig. 12). The spindle fibers disappear, and the cytoplasm becomes uniformly vacuolate. Many instances were seen in which the vacuoles tended to coalesce gradually, forming a large central vacuole (fig. 13). At no time is there any indication of a cell plate. The formation of four macrospore nuclei without the intervention of cell walls agrees with SHATTUCK'S (6) observations in *Ulmus americana*. The macrogametophyte grows and the four nuclei divide (fig. 14). The large central vacuole previously present has disappeared at the eight-nucleate stage. Three of the eight nuclei remain at each end of the macrogametophyte; the two polar nuclei migrate toward the center of the cell. These come in contact and fuse before fertilization to form the primary endosperm nucleus (fig. 15).



FIGS. 6–18.—Stages in development of macrogametophyte: Fig. 6, macrospore mother cell in autumn, early prophase; ×2000. Fig. 7, the following spring; ×2000. Fig. 8, diakinesis; ×2000. Fig. 9, heterotypic division, equatorial plate; ×2000. Fig. 10, two-nucleate macrogametophyte; ×950. Fig. 11, homoeotypic division of macrospore nuclei; ×950. Fig. 12, four-nucleate macrogametophyte; ×950. Fig. 13, same showing large central vacuole; ×950. Fig. 14, eight-nucleate macrogametophyte; ×950. Fig. 15, mature seven-celled macrogametophyte; ×500. Fig. 16, transverse section of synergid and egg; ×950. Fig. 17, chalazal end of macrogametophyte showing four antipodals; ×950. Fig. 18, mature ovule with beaklike nucellus; ×125.

The egg enlarges and assumes an ovoid form. At maturity, large vacuoles occupy its apical end; the nucleus is located in the basal portion, surrounded by dense cytoplasm (fig. 15). Adjacent to the egg are two pear-shaped synergids. A cross section through the micropylar end of the macrogametophyte (fig. 16) shows the relative



FIGS. 19–28.—Development of embryo: Fig. 19, two-celled proembryo. Fig. 20, three-celled proembryo showing apical and basal cells. Fig. 21, vertical division of apical cell. Fig. 22, transverse division of apical cell occurring before vertical division. Fig. 23, eight-celled embryo. Fig. 24, sixteen-celled embryo; multicellular suspensor. Figs. 25, 26, 27, further development of embryo and suspensor. Fig. 28, embryo showing cotyledons, epicotyl, hypocotyl, and suspensor. Figs. 19–27, ×290; fig. 28, ×105.

position of the egg and synergids. The antipodal cells vary in shape, but tend to become elongated as the macrogametophyte matures. They do not usually disintegrate before fertilization, as reported by SHATTUCK. Occasionally four antipodals are present (fig. 17), and in one instance a macrogametophyte with twelve nuclei was observed—a condition frequently found by SHATTUCK. While the

macrogametophyte is enlarging, the cells of the nucellus at the micropylar end increase in number, forming a beaklike structure (fig. 18) which extends into the micropylar region. The cells of the beak are larger and stain more darkly than the surrounding cells.

Embryo development

The zygote divides transversely to form a two-celled embryo, consisting of a small apical cell and a larger basal cell (fig. 19). A transverse division of the apical cell results in a tier of three cells (fig. 20). Usually a vertical division occurs in the apical cell before the subapical one divides transversely and vertically (fig. 21). Exceptions to this rule were observed as shown in figure 22. After the division of the subapical cell the proembryo consists of five cells, of which the two at the apical end develop into the embryo proper and the remaining three into the suspensor. Periclinal divisions (figs. 24, 25) occur in the embryo proper, cutting off the dermatogen. In consequence of further divisions, the embryo becomes a spherical mass of cells and later heart-shaped (fig. 27). The suspensor becomes multicellular. Two leaflike cotyledons are differentiated at the apex of the embryo. The epicotyl arises between the cotyledons at the apex of the embryo. The basal portion of the embryo develops into the hypocotyl (fig. 28).

Summary

1. The ovule of *Ulmus fulva* is anatropous, consisting of two integuments and a massive nucellus which becomes beaklike at maturity.

2. The archesporial cell functions as a macrospore mother cell.

3. The macrospore mother cell nucleus undergoes meiosis, forming four macrospore nuclei in a linear row.

4. No cell plates or walls are formed between the macrospore nuclei.

5. A typical eight-nucleate macrogametophyte is formed as a result of one further nuclear division.

6. The zygote, by transverse divisions, forms a row of three cells. The apical cell of this row develops into the embryo. The two basal cells form the suspensor.

The writer is indebted to Professor C. E. ALLEN for helpful suggestions and kindly criticism during the course of this study.

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HORMONES AND ROOT FORMATION

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Introduction

Beginning with SACHS (7) in 1880, investigators have explained root formation on cuttings on the basis of the accumulation of special root forming substances near the basal cut surface. Success in obtaining an active root forming substance, however, was first achieved by WENT (12) in 1929. He found that substances which were active in inducing roots on *Acalypha* cuttings diffused from leaves of *Acalypha* and *Carica papaya*, when placed with their petioles in water. Later THIMANN and KOEPFLI (9) showed that synthetic indole(3)acetic acid, a growth substance, was active in promoting root formation on pea cuttings.

Since these initial researches, the promotion of root formation by indole(3)acetic acid and other synthetic growth substances has been observed by other workers (3, 5, 10). Experiments recently reported by the writer (4) indicate that indoleacetic acid controls the movement of other substances within the plant which are necessary for root formation. Lemon cuttings treated at the base with a strong solution of indoleacetic acid developed a great number of roots at the base. If, however, the treated bases were cut off immediately after treatment and the cuttings treated again, no more roots were initiated than on untreated cuttings. This suggests that other substances necessary for root formation accumulated at the base under the influence of the original indoleacetic acid treatment, and were removed when that portion was cut off. Additional experiments have been conducted and a study has been made of the relation of the amount and distribution of auxin, and of the influence of the presence or absence of leaves on root formation.

[1] The studies here reported were made in the biological laboratories of the California Institute of Technology at Pasadena, California, and represent part of a thesis presented to the faculty of the Graduate School of the California Institute of Technology in partial fulfilment of the requirements for the degree of Doctor of Philosophy. The writer is indebted to Professor F. W. WENT for many helpful suggestions and criticisms.

Material and methods

Eureka lemon and Delicious apple stem cuttings were used as experimental plants. The cuttings, unless otherwise noted, were 12 cm. long, from mature terminal growth, and had six to eight buds 1.5–2 cm. apart. Two full sized leaves were left at the apex. The basal cut was made just below a bud. Ten to twenty cuttings were used in each experiment, and in all cases the standard error of the mean for root counts is given.

Water solutions of synthetic indole(3)acetic acid (Merck) were used in all experiments. The basal ends in some cases and the apical ends in others were placed in the test solutions to the depth of 1 cm., and were left in nearly saturated atmosphere during the treating period (20 to 40 hours as noted specifically for each experiment). On removal from the test solution, the cuttings were rinsed with tap water and the morphological bases were inserted about 3 cm. deep in sand in a sash-covered propagating frame with bottom heat thermostatically controlled at 30° C.

Chloroform extraction technique

By certain modifications in Thimann's (**8**) chloroform extraction method it was possible to extract and measure quantitatively the auxin in treated lemon cuttings. The procedure adopted in this investigation was as follows: 2.5 gm. of freshly scraped bark was immersed in a mixture of 45 cc. of Merck's reagent chloroform and 5 cc. of 1 N HCL. After 20 hours the bark was filtered off and the chloroform layer separated from the filtrate. The chloroform was distilled off, leaving an oily residue; 2.5 ml. of water was added to this residue and this was heated for one hour over a water bath at about 75° C. The water was poured off, and several dilutions were made of it for the auxin test. An agar plate (8 × 10.7 × 1.5 mm.) was added to each dilution and was left for one hour. Finally the agar plate was cut into twelve equal blocks and tested for auxin by the standard *Avena* technique (**14**).

The average curvature obtained for the twelve blocks was multiplied by the amount of the dilution of the 2.5 cc. water extract (containing auxin from a 2.5 gm. sample) and this value was in turn

divided by 2.5 to obtain "units auxin per gram sample." These units are equivalent to about 160 "*Avena* Einheit" (AE) of KOGL and HAAGEN SMIT (**6**). A control 10-unit stock solution of indoleacetic acid was tested on each day of the experiment in order to determine the variation in the sensitivity of the *Avena* test plant. The experimental determination for auxin in the samples under test was corrected accordingly.

In all probability the growth substance extracted from cuttings treated with indoleacetic acid is largely that acid. In this paper, however, the more general name, auxin, is used because a small amount of the native growth hormone, probably auxin a, was also extracted from untreated cuttings (table 1).

In several instances a second extraction of the bark was tested. The results were as follows:

Sample no.	First extraction	Second extraction
1	560 units	42 units
2	664	58
3	110	6

Since the amount of auxin in the second extraction was in all instances less than 10 per cent of that of the first, the procedure of only one extraction was adopted as it was desirable to have as simple process as possible. The results should be considered as relative only and not as representing the absolute amount of auxin in the sample.

Results and discussion

RELATION OF AMOUNT AND DISTRIBUTION OF AUXIN IN CUTTINGS TO ROOT FORMATION

LEMON CUTTINGS TREATED AT BASE.—Table 1 shows the amount of auxin obtainable from the bark at the apex and base of cuttings at different times after treatment at the base with 0.001, 0.005, and 0.02 per cent indoleacetic acid solutions. Ten leafless lemon cuttings were used in each extraction, apex extractions being made from 3 cm. of bark at the extreme apex and base extractions from 3 cm. of bark at the extreme base. Any bark in excess of 2.5 gm. fresh weight in each sample was discarded.

TABLE 1

DISTRIBUTION OF AUXIN IN BARK OF LEAFLESS LEMON CUTTINGS AT DIFFERENT TIMES AFTER TREATMENT AT BASE FOR 20 HOURS WITH WATER SOLUTIONS OF INDOLEACETIC ACID OF VARIOUS CONCENTRATIONS

Percentage concentration of indoleacetic acid	Time in respect to 20 hour treatment	Auxin in units per gram bark	
		Apex	Base
0.02	Just before	1	1
	Just after	3	680
	1 day after	1	30
	3 days after	0	4
	10 days after	0	0
	20 days after	0	4
0.005	Just before	1	1
	Just after	0	34
	1 day after	0	18
	3 days after	0	.....
	10 days after	0	1
	20 days after	0	0
0.001	Just before	1	1
	Just after	3	8
	1 day after	0	1
	3 days after	0	0
	10 days after	0	0
	20 days after	0	0
Tap water	Just before	1	1
	Just after	0	0
	1 day after	0	0
	3 days after	0	0
	10 days after	0	0
	20 days after	0	0

TABLE 2

ROOT FORMATION ON LEAFLESS AND LEAFY LEMON CUTTINGS TREATED AT BASE FOR 20 HOURS WITH WATER SOLUTIONS OF INDOLEACETIC ACID OF VARIOUS CONCENTRATIONS

Percentage concentration of indoleacetic acid	Average number of roots per cutting after three weeks*	
	Leafy	Leafless
0.02	28.6±3.8	11.0±1.0
0.005	1.7±0.6	1.4±0.3
0.001	0.8±0.4	0.6±0.1
Tap water	0.9±0.3	0.5±0.2

* Includes roots plus root primordia. Twenty cuttings in each experiment.

TABLE 3

DISTRIBUTION OF AUXIN IN BARK OF LEAFLESS LEMON CUTTINGS AT DIFFERENT TIMES AFTER TREATMENT AT APEX FOR 20 HOURS WITH WATER SOLUTIONS OF INDOLEACETIC ACID OF VARIOUS CONCENTRATIONS

Percentage concentration of indoleacetic acid solution	Time in respect to 20 hour treatment	Auxin in units per gram bark				
		March experiment		April experiment		
		Apex	Base	Apex	Middle	Base
0.02	Just before	*	2	4	3	4
	Just after	790	8	1280	†	3
	1 day after	72	20	60	4	3
	3 days after	17	3	11	0	1
	10 days after	3	0	9	0	0
	20 days after	*	*	5	0	0
0.005	Just before	*	2	4	3	4
	Just after	41	6	72	8	10
	1 day after	8	2	8	16	6
	3 days after	2	*	2	0	0
	10 days after	1	1	*	0	0
	20 days after	*	*	0	0	0
0.001	Just before	*	2	4	3	4
	Just after	12	2	8	3	4
	1 day after	3	5	2	12	2
	3 days after	2	2	0	0	0
	10 days after	2	0	0	0	0
	20 days after	*	*	0	0	0
Tap water	Just before	*	2	4	3	4
	Just after	2	2	2	1	1
	1 day after	*	*	1	0	0
	3 days after	*	*	1	1	1
	10 days after	*	*	0	0	0
	20 days after	*	*	0	0	0

* No determination made. † Below 20 units.

TABLE 4

ROOT FORMATION ON LEAFLESS LEMON CUTTINGS TREATED AT APEX FOR 20 HOURS WITH WATER SOLUTIONS OF INDOLEACETIC ACID OF VARIOUS CONCENTRATIONS

Percentage concentration of indoleacetic acid	Average number of roots per cutting after three weeks*	
	Average number	Increase over tap water controls
0.02	1.8±0.5	1.3±0.5
0.005	0.6±0.2	0.1±0.2
0.001	0.5±0.1	0
Tap water	0.5±0.2	.....

* Includes roots plus root primordia. Twenty cuttings in each experiment.

The bark of the treated base of the cuttings showed large amounts of auxin immediately after treatment, indicating that solutions of the strengths tested are actually absorbed by the cutting. The amount, however, was by no means proportional to the concentration of the solution. The stronger solutions were absorbed much more readily than the weaker ones. The 0.02 per cent solution was only four times as strong as the 0.005 per cent solution, yet about twenty times as much auxin was recovered from the former. Likewise over eighty times as much was extracted from the cuttings treated with 0.02 per cent as from the 0.001 per cent cuttings, while the difference in concentration of solution was of the order of one to twenty.

The relatively large amounts of auxin occurring in the base of the cuttings immediately after treatment did not persist long, about 95 per cent disappearing during the first day in the case of the 0.02 per cent treatment. The absence of any appreciable amount of auxin in the apex at any time indicates that there was no upward movement to the apex. A few extractions were made of the wood, which showed, just as with the bark samples, very little auxin in any part of the cutting other than that immersed in the solutions. The fact that all extractions were made on leafless cuttings almost completely eliminated transpiration as a factor affecting upward transport in the xylem. As yet no extensive data have been obtained on distribution of auxin in leafy cuttings.

Root counts on both leafy and leafless cuttings treated with these same solutions are given in table 2. It is seen that with both leafless and leafy cuttings, only the 0.02 per cent solution was effective in root formation, the 0.001 and 0.005 per cent solutions being no more effective than tap water. Since more auxin was found in the base of cuttings treated with 0.001 and 0.005 per cent solutions than in cuttings treated with tap water, it appears that some factor other than auxin was limiting root formation. In the case of treatments with the 0.02 per cent solution, this other factor was apparently no longer limiting since a large number of roots were initiated. One possible explanation for this response is that the strong indoleacetic acid solution mobilized the other factor.

LEMON CUTTINGS TREATED AT APEX.—When the cuttings were treated at the apex with the 0.001 and 0.005 per cent solutions there

was apparently a downward transport of auxin, as indicated by the recovery of moderate amounts of auxin in the base as compared with very little in the base of control samples (table 3). This accumulation of auxin in the base was greater after treatment with the 0.005 than with the 0.001 per cent solution. In the apical treatments with the 0.02 per cent solution, a moderate amount of auxin was recovered from the base samples in one experiment (more than for the 0.005 per cent solution) but very little in the other experiment (less than for the 0.005 per cent solution). The amounts recovered in both cases were extremely small as compared with the amount occurring in the apex. Apparently applying high concentrations to the apex brings about some abnormal condition in the tissue at the point of application, for after the high level of auxin is quickly reduced, the remaining small amount is retained in the apex for a period of at least 20 days even when other regions of the cutting are devoid of auxin in measurable amounts. Since this fact was not observed in connection with the 0.005 and 0.001 per cent solutions, it is suspected that the normal polar transport is upset by the use of high concentrations, and that in this case transport is influenced by the condition of the cutting at the time of the treatment.

It is seen from table 4 that very few roots were formed at the base on any of the cuttings treated at the apex. The failure of the 0.02 per cent solution to give the pronounced response obtained by basal treatments is accounted for by its limited downward transport in lemon cuttings. In some instances treatment at the apex (with 0.02 per cent or higher concentration) caused root formation both at the apex and base. This condition occurred only occasionally, however, but would perhaps have been more pronounced had temperature and moisture conditions been as favorable for root development at the apex as at the base. On the basis of relative auxin content, one would expect a greater number of roots to appear at the apex than at the base.

APPLE CUTTINGS TREATED AT BASE.—So far all attempts to root Delicious apple cuttings with indoleacetic acid treatments have failed. Cuttings of various kinds and conditions taken at every month of the year were used, and not one root was observed. The question then arose as to whether apple cuttings failed to absorb the acid or contained some mechanism whereby the substance was de-

stroyed on being absorbed. In an attempt to solve this problem, apple and lemon cuttings were treated in the usual manner with a 0.02 per cent indoleacetic acid solution for 20 hours, and auxin extractions were made of the bark of both the lemon and apple: (1) immediately after the treatment, (2) the next day, and (3) three days after the treatment. The results given in table 5 show that

TABLE 5

COMPARISON OF AMOUNTS OF AUXIN RECOVERABLE FROM BARK OF LEAFLESS LEMON AND APPLE CUTTINGS AT DIFFERENT TIMES AFTER TREATMENT AT BASE WITH 0.02 PER CENT SOLUTION OF INDOLEACETIC ACID FOR 20 HOURS

Month treated	Time after treatment	Lemon		Apple	
		Auxin in units per gram of bark		Auxin in units per gram of bark	
		Apex	Base	Apex	Base
January.....	7 hours	12	89	18	92
March.....	Immediately	4	680	4	800
	1 day	1	20	2	232
	3 days	0	4	1	7

there is very little difference in the amounts of auxin recovered from the lemon and apple cuttings. Failure of the apple to respond to indoleacetic acid therefore appears to be due, not to excessive destruction of the substance or to a failure of the cuttings to absorb it, but to causes other than a lack of the acid. One possible explanation is that apple cuttings are lacking in certain internal substances which are present in lemon cuttings and which are necessary for root formation. Evidence that internal substances are involved in the rooting of lemon cuttings by means of indoleacetic acid is presented in the following experiments.

Effect on root formation of cutting off the treated base and treating the new base

The possible role of the strong solution (0.02 per cent) of indoleacetic acid in causing root formation on lemon cuttings is seen from a study of the results given in table 6. Cuttings treated at the base

with a 0.02 per cent solution for 20 hours produced 11.7 ± 1.0 more roots per cutting than did the tap water controls. Cutting off the treated base destroyed the effect of the treatment, and treating the new base for 20 hours with 0.02 per cent solution caused only 5.8 ± 1.2 more roots than on the controls, or about half as many as found on treated cuttings without subsequent treatment. Excising the treated base likewise destroyed the effect of a 40 hour treatment

TABLE 6

EFFECT ON ROOT FORMATION OF EXCISING THE TREATED BASE AND TREATING THE NEW BASE

EXPERIMENT NO.	INITIAL TREATMENT	SUBSEQUENT TREATMENT	AVERAGE NO. OF ROOTS PER CUTTING AFTER THREE WEEKS*
A.....	0.02% indoleacetic acid for 20 hours	None	12.6±1.8
B.....	"	Base cut off	1.1±0.3
C.....	"	Base cut off and new base treated with 0.02% indoleacetic acid for 20 hours	6.8±0.7
D.....	Tap water for 20 hours	None	0.9±0.3
E.....	0.02% indoleacetic acid for 40 hours	None	17.5±1.9
F.....	"	Base cut off	0.4±1.5
G.....	"	Base cut off and new base treated with 0.02% indoleacetic acid for 20 hours	1.1±0.3

* Twenty cuttings in each experiment.

with 0.02 per cent solution, but treating the new base for 20 hours with 0.02 per cent solution gave no more roots than when not treated. Thus the number of roots obtained after cutting off a treated base and re-treating the new base depends on the length of the initial treating period. Cuttings with an initial treatment of 20 hours gave 5.8 ± 1.2 more roots than controls, while those with an initial treatment of 40 hours gave no more roots than the controls. In order to insure equal amounts of stored foods and other substances, the final length of the cuttings in all experiments was made the same. Cuttings in experiments B, C, F, and G (table 6) were

made about 3 cm. longer in the beginning to allow for the removal of 3 cm. of the stem at the base after the initial treatment.

These results are in agreement with earlier work by the writer (4), and are explained by assuming that the indoleacetic acid applied at the base in strong solution causes the downward transport of a substance (or substances) called rhizocaline,[2] which is necessary for root formation. These substances apparently accumulate in the base of the cutting under the influence of indoleacetic acid, and cutting off the treated base removes them from the cutting. The fact that there were 12.6 roots per cutting for a regular 20 hour treatment and 6.8 roots when the 20 hour treated base was cut off and the cutting re-treated 20 hours suggests that about twice as much rhizocaline moved to the base during the first 20 hours of treatment as during the second 20 hours. Also the failure to obtain roots by re-treating 40 hour-treated cuttings indicates that perhaps the rhizocaline movement to the base in this particular experiment was complete after 40 hours.

A similar conclusion regarding the role of an internal factor in root formation was reached by WENT (13) from experiments with pea cuttings. He found that, if a cutting were divided into a number of sections and each treated with a high concentration of auxin, the sum of the numbers of root primordia formed was about the same as on an intact cutting so treated. This suggests that the number of primordia is determined by internal substances, which limit root production even though auxin is in excess. The bulk of the primordia were on sections some distance from the apex; therefore they must have contained more of the internal substances. When auxin was applied to the apex of the uppermost one-eighth of a cutting, only about seven primordia were formed at the top, but when applied to the apex of the intact cutting, thirty primordia were formed at the top. The auxin therefore appears to have mobilized some other substance from the lower parts of the cutting.

Although the results of experiments on lemon suggest that an important function of indoleacetic acid in root formation is to control

[2] The term rhizocaline was first used by BOUILLENNE and WENT (2) in referring to naturally occurring root-forming substances. The same meaning is implied in the use of the term in this paper.

the transport of rhizocaline, it does not preclude the possibility that the acid may, for instance, also react with or activate rhizocaline to cause differentiation of the root meristem. Some recent experiments by WENT (unpublished data) with pea cuttings suggest that indoleacetic acid does play a dual role in root formation by first mobilizing rhizocaline and then reacting with it.

INFLUENCE OF LEAVES ON ROOT DEVELOPMENT

Since the results just presented suggest that indoleacetic acid caused the downward transport of rhizocaline, it was thought that

TABLE 7

EFFECT OF LEAVES ON ROOT FORMATION ON LEMON CUTTINGS TREATED AT BASE FOR 20 HOURS WITH 0.02 PER CENT INDOLEACETIC ACID SOLUTION

LOT	TIME LEAVES REMOVED	AVERAGE NUMBER ROOTS PER CUTTING AFTER THREE WEEKS*		
		ROOTS	ROOT PRIMORDIA	TOTAL
H.....	Before	4.3±0.9	6.8±1.2	11.1±1.4
I.....	Immediately after treatment	5.3±1.3	23.3±3.9	28.6±3.8
J.....	Twenty hours after treatment	7.1±1.2	17.0±2.1	24.1±2.2
K.....	One week after treatment	11.3±1.0	22.5±3.6	33.8±3.5
L.....	Leaves not removed	13.0±0.6	10.8±1.9	23.8±2.1

* Ten cuttings in each experiment.

possibly this substance came primarily from the leaves. If the acid causes the rapid transport of this substance from the leaves to the base of the cutting, removing the leaves shortly after treatment should have no effect on the number of roots formed, as the rhizocaline will already have moved to the base. The light intensity in the propagating frames was so low that it is doubtful whether much assimilation occurred in leafy cuttings after being placed in the frame.

With these considerations in mind, an experiment was conducted whereby leaves were removed from lemon cuttings before treatment and at various times after treatment. The results are given in table 7. It is noted that the number of fully developed roots increased

with the time that the leaves were left on the cuttings. When root primordia were included, however, the total number of roots initiated for all lots which had the leaves on at the time of treatment (I to L inclusive) was found to be roughly the same. It is true that there is a wide range in the values for these four lots, which no doubt is due to the difficulties involved in making an accurate determination of the root primordia; but they show no definite trend in relation to the time the leaves were removed, and all show a significantly greater number of roots initiated than lot H which had no leaves at the time of treatment.

These results indicate that leaves do furnish substances necessary for root formation, because all cuttings with leaves intact during the treatment showed a significantly greater number of roots than cuttings with no leaves. These results are substantiated by similar results from many other experiments with lemon cuttings. Those roots which are initiated on treated leafless cuttings are accounted for by the trapping of rhizocaline in the cutting at the time of taking it. It seems likely that in the intact plant rhizocaline is synthesized in the leaves, and moves basipetally in the twigs and branches to the roots. At the time of taking the cutting, therefore, the stem tissue as well as the leaves will contain rhizocaline.

The fact that the presence of leaves after the 20 hour treatment with indoleacetic acid had no further influence on the number of roots initiated substantiates the view that the acid causes the rapid downward movement of rhizocaline. The observation, however, that the presence of leaves after the treatment does influence the number of root primordia to elongate indicates that in this case we are concerned with substances coming from the leaves necessary for the outgrowth of root primordia. This substance (or substances) is apparently transported slowly to the base, because removing the leaves after one week gave marked reduction in the number of developed roots. Also it has been repeatedly observed that there is a significant increase in the number of developed roots on treated leafy cuttings between three and six weeks. This would be expected on the basis of the data in table 7, which show an average of 10.8 ± 1.9 root primordia per cutting still present in the base of treated leafy lemon cuttings after three weeks. On the other hand there was never a

significant increase in number of visible roots between three and six weeks and leafless cuttings, although histological examinations showed a great number of root primordia present. It appears that the leaves on lemon cuttings furnish substances necessary for further growth of root primordia, and that these substances are different from root-forming substances.

Experiments similar to the preceding have been conducted on chrysanthemum cuttings and the results are presented in table 8. In these experiments the cuttings were not examined for root primordia so it is not possible to segregate the separate actions of root-forming and root-elongating substances. Nevertheless the data show the same relation between the time leaves were removed and the number of visible roots as in the case of lemon cuttings.

It is noted from table 8 that the indoleacetic acid treatment caused an injury to the base of cuttings without leaves while no injury was observed on cuttings with leaves. With lemon also it has frequently been noticed that leafy cuttings will tolerate a stronger treatment than leafless cuttings. At present no explanation can be offered for these observations.

Attempts to extract rhizocaline from leaves

Chloroform extracts of leaves of a number of different plants have failed to show any response other than that of promoting growth of the *Avena* coleoptile, and therefore probably contained the naturally occurring growth hormone, auxin a. The growth hormone apparently is not rhizocaline, however, because Delicious apple leaves and bark were found to contain as much of it as the lemon. Thus it seems likely that factors other than auxin are involved.

A second attempt to extract rhizocaline was made by placing petioles of cut-off leaves of several different plants in a strong solution of indoleacetic acid, the idea being that the acid would mobilize the rhizocaline from the leaf. The results of one experiment with lemon leaves showed that the diffusate into the acid appeared to increase slightly the root-forming activity of the indoleacetic acid solution. More experiments must be conducted to determine whether the difference is real. All types of leaves are apparently not adapted to this technique because the diffusate from *Lantana* leaves definitely

destroyed the effectiveness of the indoleacetic acid solution, while fig leaf diffusates reduced the activity of the acid to half its normal value. Enzymes set free from the cells at the cut surface of the petiole may have destroyed the acid in a manner similar to that ob-

TABLE 8

EFFECT OF LEAVES ON ROOTING OF UNTREATED AND TREATED CHRYSANTHEMUM CUTTINGS*

VARIETY	INDOLEACETIC ACID TREATMENT	TIME LEAVES REMOVED	TIME IN SAND (WEEKS)	AVERAGE NUMBER ROOTS PER CUTTING†	TREATED BASE INJURED
Meta Bergen	None	First day after treatment	2	0	
		Third day after treatment	2	4.5±1.0	
		Not removed	3	11.3±1.6	
	0.01% for 18 hours	First day after treatment	3	6.3±1.3	
		Third day after treatment	3	6.9±0.8	
		Not removed		10.2±1.5	
Willard's Bronze....	0.01% for 18 hours	Before treatment	3	2.2±0.9	+
		Third day after treatment	3	15.5±1.2	−
		Not removed	3	26.3±1.6	−
	Tap water for 18 hours	Before treatment	3	0.5±0.3	−
		Not removed	3	0.3±0.05	−

* Cuttings placed in sash-covered propagating frame without bottom heat.
† Ten cuttings in each experiment.

served by VAN OVERBEEK (11) for sections of bases of corn mesocotyl.

Conclusive evidence of the existence of rhizocaline and other naturally occurring substances, as postulated in this paper, awaits actual extraction. Further work is now in progress. At the same time various combinations of sugars, amino acids, and vitamins are being tested in connection with and following the indoleacetic acid treatment. It is suspected that one of the substances necessary for the outgrowth of the root primordia is vitamin B_1, since this substance has been found by BONNER (1) to be necessary for the growth of excised pea roots.

Summary

1. Portions of cuttings, some of which had been treated at the apex and some at the base with indoleacetic acid, showed large amounts of auxin in the bark immediately after treatment, but about 90 per cent of the auxin disappeared during the first day.

2. Very little auxin was recovered from the bark at the apex of cuttings which had been treated at the base with 0.001, 0.005, and 0.02 per cent solutions of indoleacetic acid.

3. Moderate amounts of auxin were recovered from the base when the cuttings were treated at the apex with these solutions; however, with the 0.02 per cent solution the amount recovered at the base was small as compared with that at the apex.

4. The 0.02 per cent solution applied at the base of lemon cuttings was the only treatment of those tested that gave significant increase in root formation on lemon cuttings over controls.

5. There was little difference in the amount of auxin recovered from treated apple and lemon cuttings, yet the apple cuttings did not form roots. It is assumed that apple cuttings are lacking in certain internal substances necessary for root formation.

6. Experiments involving removal of the treated base and treating of the new base indicate that the action of indoleacetic acid in root formation is primarily the mobilization of naturally occurring root-forming substances.

7. Evidence is presented which indicates that leaves of lemon cuttings supply a substance necessary for the differentiation of the root primordia and another for the outgrowth of these primordia. The former appears to be transported rapidly to the base under the influence of indoleacetic acid while the latter is transported slowly to the base.

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CORRELATIVE EFFECTS OF ENVIRONMENTAL FACTORS ON PHOTOPERIODISM[1]

KARL C. HAMNER

In 1920 GARNER and ALLARD (**7, 8**) announced the results of experiments and observations on the responses of plants to alternating periods of light and darkness, or variations in the length of day and night. The term photoperiod was adopted to designate this periodic alternation as an environmental factor, and the term photoperiodism to designate the responses on the part of the plant.

What constitutes a long day or short day may be arbitrarily fixed by an investigator, but in dealing with the response of flowering the concept of GARNER seems particularly pertinent. He states (**11**): "For those plants which are more sensitive to the length-of-day factor, there exists a fairly definite critical light period which constitutes the dividing line between day lengths favorable to flowering and fruiting and those tending to produce a purely vegetative type of activity. In the short-day group flowering is initiated by day lengths shorter than the critical, and in the long-day group flowering is initiated by day lengths in excess of the critical. Commonly among annuals and herbaceous perennials the alternative vegetative stage of the short-day type is characterized by indeterminate elongation of the axis, while a prominent feature of the vegetative stage in the long-day type is embodied in a leaf-rosette form of growth without significant stem elongation. To determine the classification of a given plant it will usually suffice to observe the nature of the response to a day length less than and to one greater than the critical light period. The essential characteristic of the less sensitive or indeterminate group of plants is that they possess no clearly defined critical light period."

It is important to keep in mind the indeterminate group, since a

[1] Invitation paper read at the joint meeting of Section G, American Association for the Advancement of Science, the Botanical Society of America, American Phytopathological Society, American Society of Plant Physiologists, Mycological Society of America, at Indianapolis, December 28, 1937.

great many attempts have been made to apply directly the results of experiments on that group to an analysis of the results of experiments with the other two groups. As an example, the endeavors to apply the chemical results found associated with flowering and fruiting of the tomato—an intermediate or day-neutral plant—have failed to account for the initiation of flower primordia, although the data apply very well to fruit setting and fruit development.

Although in the literature most emphasis has been placed upon the flowering responses of plants to photoperiod, photoperiodism involves any response of a plant to changes in photoperiod. In their earliest papers, GARNER and ALLARD pointed out that vegetative extension, tuberization, and other responses are definitely similar. They state (9), "The available data indicate that there is an optimal light period for tuber formation just as there are optimal periods for stem elongation and for sexual reproduction. The highest proportionate production of tubers occurs with a light period which admits of only very limited stem growth. The absolute maximum production of tubers, however, is obtained with an intermediate light period." . . . . "Within the range of duration of light exposures which will admit of alternative forms of expression there are in most cases certain exposures favorable only to sexual reproduction and other exposures favorable only to vegetative activity. That is, there are more or less definite optima in light exposures for the two alternative forms of expression. The question arises as to the response of the plant to light exposures intermediate between those specifically favorable to the two types of activity. In reality the effects of these intermediate exposures are important and are both quantitative and qualitative in character."

Much confusion has arisen in discussions involving a quantitative delimitation of the vegetative, flowering, fruiting, or reproductive condition in relation to environmental factors or internal condition, especially chemical composition, because of the failure of specific designation of the material investigated. Much of what has been said concerning the effect of photoperiod on the reproduction of angiosperms is not easy to integrate, especially since the angiosperms are a group in which it is particularly difficult to set a limit within which plants are distinctly vegetative or distinctly reproductive. If,

as is often done, gametic differentiation and gametic union are considered the final criteria for determining sexual reproduction, then much of what has been written about blooming, fruiting, and so forth has no particular significance as sexual reproduction, but must be considered only as a correlated condition.

In attempting an integration of several factors affecting photoperiodism or determining the role of photoperiod as one of several factors influencing plant behavior and development, it is essential first of all that the vegetative states and the flowering and reproductive states be thought of, not as antagonistic functions, but as correlated conditions which constitute a range of qualitative expression. An apple tree of moderate size may produce one blossom cluster, or a hundred, or a hundred thousand clusters. In any of these cases the tree may be considered as being in flower. Qualitatively the three situations are alike if blooming of the tree is made the basis of classification, but the quantitative differences are very great and so too are the physiological conditions within the tree as a whole.

It may be difficult qualitatively to differentiate between a vegetative and a reproductive plant. The transition from floral leaf to vegetative leaf may be very gradual. It would easily be possible to arrange a closely graded series of structures which all agree are vegetative, through those which are in close association with the sexually reproductive structures, to the sexually reproductive structures themselves, that is, the gametes. It is better therefore to speak of the flowering or non-flowering condition, rather than of the reproductive condition, unless such reproduction actually involves gametic differentiation. That such reference has a very real point will be brought out later in relation to the failure of pollen grains or gametophytes to come to maturity under certain conditions of photoperiod and mineral nutrient supply, although apparently fully developed flowers may be produced. There is need for precise records showing the influence of photoperiod upon (1) the initiation of flower primordia as contrasted with (2) its influence upon the development of flowers, (3) its influence upon the development of fruits, and (4) its influence upon the development of seeds.

In this connection it is important to reconsider GARNER'S concept

of the critical period previously given. It is possible for many plants to differentiate floral primordia at day lengths both longer and shorter than the critical period, but the subsequent development of these varies greatly. An example is the Biloxi soy bean, a short-day plant (**7, 9**). On plants maintained at or just below the critical period, approximately a 13-hour day, a condition approaching everblooming is attained, the primordia develop into flowers in which pollination takes place, and fruits and seeds develop. If the plants are maintained at day lengths appreciably shorter than the critical, at exposures of 8 hours or less, the primordia may develop into flowers which are small, often minute; but it is only seldom that full sized pods are formed, and few seeds are produced. This condition is associated with marked suppression of vegetative extension of the plant as a whole as compared with plants which have differentiated floral primordia and have continued to be maintained at the critical period. On the contrary, on plants which are held for a few days at the critical period or slightly below it, until flower primordia have been initiated, and then transferred to a photoperiod longer than the critical, at least some of the primordia may continue to develop into flowers which are very much larger than those on plants maintained at or below the critical period. The carpels or fruits of these may fail to develop, or develop very slowly, and often contain no seeds. Associated with this condition is a much greater vegetative extension of the entire plant, leaves, length of internodes, and diameter of the stems. Under similar conditions of photoperiods greater than the critical, the flower buds of chrysanthemum may abort completely and the plant as a whole be definitely vegetative. In the case of *Cosmos* (**2**) some of the floral primordia continue development, but the flower heads and individual flowers exhibit wide ranges in type, the corollas of some of the outer ray flowers being green and leaflike. Some of the flower heads may appear as close clusters of small green leaves. Just as in the former cases mentioned, these conditions are associated with a pronounced vegetative state of the plant as a whole, the most vegetative condition being associated with complete failure of differentiation of floral primordia. As previously indicated, it is important that at least four phases, initiation of flower primordia, development of flowers, of fruits, and of seeds,

be kept in mind as distinct segments on a graded scale, the margins of such segments intergraded but their midportions clearly recognizable. Chemical studies (**19**) have shown certain distinct correlations with the several types of development after flower primordia have been initiated, but of the substances so far determined none has been found which accounts for the initiation of such primordia. After such initiation the developmental behavior of photoperiodically sensitive plants closely parallels that of the non-sensitive, indeterminate, or day-neutral tomato.

Because of the normal shifting of the length of day in a natural environment in most latitudes, and because of the preponderance of so-called switch-over experiments under controlled environments, it is important to integrate the effect which one environment, to which a plant has been exposed, exercises when the plant as a whole, or some of its parts (**10**), is placed under another environment. This period of exposure to a given environment whose effects are carried over and continue to be manifest under a changed environment may well be referred to as an induction period (**16**). Thus the term photoperiodic induction, or after-effect, would mean the carry-over effect exhibited when a plant is transferred from one photoperiod to another. Some short-day plants will initiate flower buds if given as few as three short days and subsequently grown on long day. Thermo-induction, the carry-over effect of temperature as an environmental factor, has long been recognized but more recently has been re-emphasized (**24, 6, 18**). Similarly one might well consider a chemo-induction period, as is well illustrated by plants when grown under varying planes of nutrient supply. As a specific example, some plants once brought to the fruiting condition with a relatively low plane of nitrogen supply will continue to fruit at a plane so high that, if initially supplied to them, fruiting would not have taken place. This terminology is largely a matter of breaking up into somewhat more specific verbiage the general term of KIDD and WEST, physiological predetermination (**13**), and would include much that is now listed under the term yarovization or vernalization. Despite the terminology, relatively little is known concerning the precise method of operation of these inductive influences in relation to subsequent developmental patterns or behavior. The existence

of such possible carry-over effects should be recognized. Failure to take them into account has led to some wholly inadequate and erroneous interpretations of supposed immediate effects of the environment, which effects were in reality the expression of a reaction to the environment to which the plants had been exposed previously as well as the immediate one.

Since the discovery of the effects of photoperiod upon flowering and fruitfulness, many efforts have been made to correlate length of day and nutrition. Most of these attempts have been designed to see whether changes in photoperiod affect the carbohydrate-nitrogen relations of the plant in sufficient degree to explain the initiation of the flowering state. In general, this has been found not to be the case. Most short-day plants growing on a long day cannot be induced to form flowers by wide variations in the amount of nitrogen supply or by conditions which favor or inhibit carbohydrate synthesis. Even though the light intensity during any given photoperiod be greatly reduced, as is experienced in the winter months as compared with those of summer, the time required for floral initiation of any given variety of plant is not greatly changed, although the number of floral primordia initiated may be considerably fewer. Also the subsequent development of such primordia is dependent upon the intensity of illumination during the photoperiod. Very commonly floral primordia initiated during a long or a short photoperiod of low intensity will fail to continue to develop into flowers or fruit, whereas if the light intensity is high during all or a considerable portion of the photoperiod, they will continue to develop.

Even in the early work on the carbohydrate-nitrogen relationships associated with blooming and fruiting of the tomato (**15**), emphasis was placed upon their effect upon fruitfulness rather than on the initiation of flower primordia. It was stated that "the conditions for the initiation of floral primordia and even blooming are probably different from those accompanying fruit setting. The greatest number of flowers are produced neither by conditions favoring highest vegetation nor by conditions markedly suppressing vegetation." Unfortunately in many subsequent attempts to apply those findings to other plants, these distinctions have been largely overlooked.

Lacking as yet a determination of a specific substance or substances, on the basis of which the initiation of floral primordia can be accounted for, it is possible to say only that in many plants the initiation of the flowering condition is determined by the day length. Evidence of a correlation between chemical composition and type and character of subsequent development of such primordia after they have been initiated is far more clear and direct (**19**).

It has been found that nitrogen deficiency induced the earing of barley plants, a long-day type, growing on a 9-hour day, whereas with an abundance of nitrogen the plants failed to produce ears (**3**). A 9-hour day would be considered below the critical day length of barley, and hence, if photoperiod were the only factor to be considered in blooming, the plants should not have blossomed. There has been at least one report (**1**) that nitrogen deficiency could affect the time of flowering of a short-day plant growing on a short day and a long-day plant growing on a long day, but the reports demonstrating that the plane of nitrogen nutrition may be substituted for changes in photoperiod in inducing flowering in long and short-day plants have been few.

It remains to be shown whether changes can be effected if plants are grown at their critical photoperiod. It is certain that short-day plants may be affected when grown on day lengths longer than the critical after an induction period; the longer the length of day above the critical period, the more striking the effect. Many of the experiments which have dealt with this problem have involved, consciously or otherwise, plants which have been subjected to an induction period consisting of a few short days at the critical period or below it. During this period flower primordia were initiated and the plants then transferred to day lengths longer than the critical period. Under such circumstances the plants generally flower and often produce fruits, the extent of flowering being dependent upon the length of day above the critical period, the greater this lengthening the more vegetative the plant and the less the amount of flowering or seed production. As has been pointed out, if short-day plants are grown at day lengths appreciably below the critical period, not only the extent of flowering but also their vegetativeness is concurrently decreased. At photoperiods slightly greater than the critical period,

flowering and fruiting may be increased in association with an increased condition of vegetativeness; but if the length of the photoperiod is greatly increased, flowering and fruiting are decreased along with an increase in vegetativeness. Experience has shown that the addition of relatively large increments of nitrogen to plants below the critical period has little or no effect on the behavior of the plant, but above the critical period the addition of nitrogen does increase the degree of vegetativeness and the total net weight of the plants (20). Within certain ranges the number of flowers and fruits produced may also be augmented.

Because of the decided differences in behavior of plants at photoperiods above and below the critical period in relation to their response to nitrogen supply, it would be worth while to attempt some more specific evaluation of the critical period in terms of physiological response or chemical behavior. Thus controlled photoperiods offer one of the most valuable tools for investigating the whole problem of metabolism, not only in relation to flowering and fruiting but in relation to the entire range of vegetative response, either as such alone or in association with the differentiation of floral primordia, and their possible consequent development. What is now termed the critical period may well mark a rather definite point of change in physiological behavior, and should therefore furnish a valuable clue for the selection of materials on which to base physiological and chemical analyses.

Although the general qualitative response of the plant seems to be conditioned primarily by photoperiod, in general the quantitative range of expression of this response is influenced by the plane of nitrogen supply. For example, *Xanthium pennsylvanicum*, the cocklebur, will flower in a short time if grown on a short day and remain vegetative on a long day. Restriction of the nitrogen supply will not induce flowering in the plants being subjected to the long day, nor will an abundant supply inhibit flowering of those on the short day. Subsequent to a photo-induction period, however, the plants on a long day with abundant nitrogen grow more rapidly and produce more flowers and fruits which come to maturity at a slower rate than if the plane of nitrogen supply is low. The same general type of response is manifested by the plants maintained on a short

day, except that all the plants mature more rapidly than those on the long day. The greatest numbers of fruits per plant are produced under conditions of the short day with a comparatively high nitrogen supply.

Salvia splendens (**20**) when grown on a 17-hour day with an abundance of nitrogen will produce abundant vegetative growth but no flowers. If a plant which has been growing for some time under such conditions is transferred to a day length of about 14 hours, it will produce flowers. The number of flowers produced under such conditions is greatly influenced by the supply of nitrogen, however, plants receiving little or no nitrogen developing many more flowers than plants receiving a plentiful supply. It may be that the 14-hour day is near the critical period for *Salvia*, and because of this fact the supply of nitrogen produces a marked effect.

Work has been done relating to a more critical dissection of these results in terms of chemical composition and in terms of cytological detail. In some plants, changes in photoperiod affect the capacity of the plant to assimilate nitrogen (**20**). Thus certain short-day plants growing on a photoperiod shorter than the critical may take up large quantities of nitrates which are not assimilated but accumulate in the plants as nitrates. Possible changes in the carbohydrate-nitrogen relations have been correlated more directly with the development of flowers and fruits than with the differentiation of flower primordia (**19**). Cytologically (**12**) it has been shown that in the tomato, a day-neutral form, plants deficient in carbohydrates and having a great abundance of nitrogen were unfruitful, not primarily because flowers failed to differentiate but because no functional pollen grains developed. If plants of the cocklebur are given a photoinduction period of several days and are then divided into four lots, short-day low nitrogen supply, short-day high nitrogen, long-day low nitrogen, and long-day high nitrogen, all four lots will flower but each lot shows a characteristic development.

The short-day low nitrogen plants develop relatively few flowers, either staminate or pistillate, but the anthers contain the highest percentage of fertile pollen grains of any of the lots.

The short-day high nitrogen plants develop more staminate

flowers than those on low nitrogen, but the stamens contain a higher percentage of sterile pollen grains.

The long-day plants on low nitrogen develop fewer flowers, both staminate and pistillate, than similar plants on high nitrogen, but the flowers mature more rapidly. The number of defective pollen grains is about the same as the short-day high nitrogen plants.

The long-day plants with high nitrogen develop the greatest number of flowers and the greatest length of time is required for the fruits to reach maturity. The number of defective pollen grains is about the same as in the other lots, except the one on short day and low nitrogen, which it will be recalled had by far the greatest number of fertile grains.

While the number of reports showing that nutrition can substitute for or nullify the effects of photoperiod are decidedly limited, the claims that temperature can do this are many. Plants such as stocks (**21**), beet (**6**), and celery (**24**) tend to be strictly vegetative if grown continuously at relatively high temperatures, but at relatively low temperatures they flower readily and develop seeds. A combination of these two conditions may be made if plants are grown for a time at a high temperature, then placed for a few days at a relatively low temperature which serves as a thermo-induction period, and then are returned to a high temperature. Under such circumstances, either floral primordia are initiated during the induction period or the character of the plant is so changed that blooming, and frequently fruit setting and seed formation, take place at the higher temperature. Depending upon the length of the induction period and the temperatures maintained after the induction period, a more or less intergraded series of responses from strict vegetativeness to a combination of vegetativeness, flowering, and fruiting in all degrees may be manifested. To give a specific example, stocks remain indefinitely in the vegetative stage when exposed continuously to temperatures around 70° F. If, however, a plant is given a thermo-induction period of a few days at temperatures approximating 50° F. and is then returned to the higher temperature, in a short time flowers and fruits are produced. This performance is characteristic of many plants, after they have undergone thermo-induction. There are also types of plants, such as lettuce (**25**), which undergo thermo-

induction at relatively high temperatures. The short exposure at a high temperature will exert a carry-over effect causing the plants to flower when subsequently grown at a low temperature.

It has been demonstrated that many plants which exhibit striking photoperiodism show little photoperiodic sensitivity after a thermo-induction period, usually flowering more rapidly the longer the light period. The fact that a plant may be thermoperiodic need not mean that it may not also be sensitive to photoperiod. The critical work that has been done on the beet (**6**) illustrates this point. If beets are grown at temperatures between 50° and 60° F., all the plants produce flowers in a short time. If they are grown continuously at temperatures fluctuating between 60° and 70° F., they will produce flowers provided they are exposed to a long day and not if they are exposed to a short day. If they are grown continuously at 70° F. or above, they will not produce flowers at any day length. Some varieties of soy beans, beet, and *Rudbeckia*, grown under precisely controlled conditions of temperature and light (**23**), have different quantitative responses to photoperiod when grown at different temperatures. Thus an increase in temperature somewhat decreases the range of critical day length for Biloxi soy beans, a short-day plant, and *Rudbeckia*, a long-day plant. With the sugar beet, also a long-day plant, an increase in temperature increases the range of critical day length.

Much confusion has arisen in discussions attempting to establish a direct correlation between the behavior of temperature sensitive plants and their chemical composition, because of failure to differentiate between qualitative and quantitative behavior. Although many plants require a certain set of conditions to bring about induction of the flowering condition, it seems generally true that, after induction has been completed, flowering and fruiting may be accelerated by a different set of conditions. Thus most plants which have undergone thermo-induction at a relatively low temperature will flower and set fruit much sooner if the temperature is then raised following the induction period (**17**). The sugar beet, as produced in southern Utah, following the cold induction period of the winter season, will flower abundantly with increased day length and temperatures which may range well above 70°. Increased increments of

nitrogen supply given following such an induction period do not inhibit blooming but result in greatly increasing the numbers of flowers and seeds produced (**22**). It has been suggested (**17**) that sexual reproduction in winter and spring wheats is not dependent upon a critical temperature or a critical light period, but the time at which it occurs is influenced greatly by both these environmental factors. Winter wheats develop flowers most rapidly if they grow under conditions of increasing day length and increasing temperature, after initial stages of short-day length and low temperature. Instead of considering winter wheats as short-day, long-day, low temperature, high temperature plants, their behavior can be harmonized with others just mentioned by regarding them as plants which respond to induction periods of low temperature and short days. Their quantitative yields would then be somewhat proportional to the degree of higher temperature, longer light period, and increased nutrient supply to which they were subjected after the induction period. Spring wheats apparently do not require or at least do not respond to the same type of induction periods.

Several investigators have suggested that during photoperiodic induction there is manufactured in the leaves of plants a flower-forming hormone, tentatively called florigen, which is transmitted to the meristems causing them to differentiate flower primordia. Spinach (**14**), a long-day plant, will not respond to long day if only a small portion of the plant near the growing point receives long day and the rest of the plant is left on a short day. The growing point will respond only if an appreciable leaf area is included in the treatment. Investigators in Europe (**4, 5**) have performed a variety of experiments which also indicate that the leaves are the organs which are sensitive to the photoperiodic stimulus. Thus if entire plants of *Chrysanthemum indicum* are grown on a long day they remain in the vegetative condition. If, however, alternate leaves on a plant grown under general conditions of a long day are exposed to a short day, all the axillary buds begin to form flower primordia, but only those in the axils of the treated leaves finally develop into flowers. Similarly, if one single leaf of such a plant is exposed to a short day, the bud or branch in the axil of that leaf will form flower

primordia, provided such an axillary branch does not have any of its leaves expanded.

The results of such experiments and the general failure to find any marked correlation between the amounts of carbohydrates, nitrogenous complexes, or similar compounds associated with initiation of flower primordia, have caused a number of investigators to postulate the presence of very minute quantities of some more recondite compound or compounds as being directly responsible for their differentiation. These are assumed to be present in the meristems, and may thus become activated or destroyed, as the case may be, by changes in photoperiod, thermoperiod, or some other environmental change. Others assume these are manufactured in the leaves, stems, or other organs of the plant and are transferred to the meristem. Most investigators prefer to withhold judgment until substances which have been isolated from the plant and synthesized in the laboratory can be applied, and the response in question secured. As yet such specific substances have not been extracted, identified, or synthesized; and even if they were now available, the method of their operation, their influence on the metabolism of the plant, and histological and structural changes following their application would still remain to be determined. What may be the relation between application of acetylene to pineapple plants (**26**) and the prompt differentiation of flower primordia and fruit development, which ensues in treated plants as compared with non-treated plants which differentiate floral primordia many weeks and even months later, is worthy of critical experimentation.

If substances can be found to which other plants respond as the pineapple responds to application of acetylene, experiments could be performed throwing much more light on photoperiodism. There may be many such substances, some acting under one set of environments, others under another, and in all possibility as many interactions as are postulated for the various growth promoting substances and hormones now so searchingly under investigation. Here would be another field open to the study of correlative factors and influences, which would after all be largely a change in phraseology rather than in point of view, in any endeavor to correlate environmental

factors and their interaction. This must be done if the present situation concerning factors influencing vegetative development, floral differentiation, fruit and seed development is to be clarified.

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GROWTH PATTERNS OF PLANTS DEVELOPED FROM IMMATURE EMBRYOS IN ARTIFICIAL CULTURE[1]

H. B. TUKEY

(WITH NINETEEN FIGURES)

Introduction

In the course of culturing immature embryos of deciduous fruits excised from the fruit at various stages in development (**23**), a definite and characteristic type of growth or growth pattern has been observed for the plants. Instead of completing the usual embryonic development from the zygote to the mature seed, as occurs on the parent plant, they have developed in culture into plantlets which exhibit a definite conformation or growth pattern apparently related to the age of each embryo when excised.

Although there are no direct references in the literature to growth patterns, as such, from immature embryos in artificial culture, most workers have called attention to some phase or another of unusual growths. HANNIG (**9**) experienced difficulty with very young embryos of *Cochlearia* and *Raphanus* and reported several anomalous growth forms. Other workers have had similar experience with very small or very young embryos, including STINGL (**17**) with embryos of *Secale*, *Triticum*, *Hordeum*, and *Avena;* DIETRICH (**3**) with *Impomea* and *Althea;* WHITE (**27**) with *Portulaca;* TUKEY (**18, 23**) with *Prunus* and *Pyrus;* and LA RUE (**11**) with *Lactuca*, *Taraxacum*, *Chrysanthemum*, and *Zea*. In all these instances immature embryos began to grow immediately without first going through the usual embryonic stages.

On the other hand LAIBACH (**12**), working with partially developed embryos of *Linum*, found that they did not germinate immediately when excised; but if they were placed in vials on cotton wadding watered by a 10–15 per cent solution of cane sugar, they developed to normal mature embryos which then germinated. Likewise KNUDSON (**10**) grew orchid embryos unremoved from the seed

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in culture and eventually these germinated and developed into normal plants. WERKMEISTER (**26**) found that if poorly developed seeds of *Iris* were placed on nutrient 10 per cent agar agar with Pfeffer's solution and 0.5 per cent glucose, they gradually imbibed water and developed so that later they could be germinated. All such plants eventually developed normally.

Mature or nearly mature seed has also produced unusual types of growth, but of a different nature from those from very young embryos. FLEMION (**4**) found that mature but non-after-ripened seeds of *Rhodotypos kerrioides* developed into plants with a dwarfish appearance, and with short, stocky hypocotyls and internodes, and small, dark green leaves. She later (**5**) reported a similar type of growth for non-after-ripened embryos of peach, apple, and hawthorn. DAVIDSON (**1**), in culturing immature peach embryos, described all plants raised in culture as abnormal and dwarfish, having small, wrinkled, and peculiarly curled leaves. VON VEH (**25**) found that seedlings of apple, pear, quince, plum, and cherry raised from non-after-ripened embryos developed into dwarf plants. LAMMERTS (**13**) secured similar results with apricot, peach, cherry, rose, and camellia; and GERSHOY (**6**) with the violet.

Material

Twelve varieties of sweet cherry (*Prunus avium* L.), five of sour cherry (*P. cerasus* L.), three of European plum (*P. domestica* L.), two of American plum (*P. americana* Marshall), thirty-two of peach (*P. persica* Batsch.), one of apricot (*P. armeniaca* L.), five of apple (*Malus domestica* Borkh.), and four of pear (*P. communis* L. and *P. communis* × *P. serotina*) have been used during five growing seasons, 1932 to 1936 inclusive.

Most of the material was from the varietal orchards of the Experiment Station at Geneva, New York, at which the work was carried on. Fruits for dissection were gathered fresh as needed. For comparative purposes fruits were also secured from Youngstown in Niagara County, New York,[2] and from Athens, Georgia.[3] Shipments from Youngstown arrived in 18 hours in good condition for dissec-

[2] Supplied through the courtesy of Dr. W. S. REED.

[3] Supplied through the courtesy of Dr. H. J. HARROLD.

tion. Shipments from Athens, Georgia, consisted of fruiting branches carefully packed in damp moss wrapped in wax paper, the cut ends of the branches being set in a damp sponge of moss. Packed in this way, twenty shipments of five varieties of peaches (covering a range of 49 days in season of fruit ripening), made at weekly intervals from full bloom to fruit ripening, were received in fresh condition within 36 hours of gathering. Material from all three sources was equally viable.

Embryos were dissected at intervals during the growing season, from April to October, covering the stage from zygote to seed maturity. In all, more than 20,000 individual cultures have been made.

Methods

Two procedures of culture have been employed: (A) using a disinfectant; (B) using aseptic conditions. The method involving a disinfectant (**18**) consisted in gathering the fruit, washing it, and opening it in the laboratory under approximately sterile conditions. The embryos were then submerged for 5 minutes in calcium hypochlorite solution prepared according to WILSON'S formula (**31**). It contains almost exactly 20,000 p.p.m. of chlorine and has proved most satisfactory. Washing with sterile water following the treatment has proved of no advantage and increases the chances for contamination.

When desirable to avoid the presence of disinfectant in contact with the material or the media, fruits were washed in a 0.1 per cent solution of mercuric chloride, and all operations, including transfer, were done in a transfer room. In some instances, with very young embryos 0.16 mm. in length, dissections were made in broth of the same concentration as the nutrient media upon which the cultures were subsequently grown. That is, in making up the media, several test tubes of solution were set aside and sterilized in which to dissect material later to be cultured in the same media. Such cultures were made on culture micro slides, in hanging drops, and in yeast culture chambers on micro slides, in addition to the larger containers described in following paragraphs. With careful attention to technique, complete freedom from contamination may be secured without recourse to disinfecting agents.

In transferring the embryos from the calcium hypochlorite solution, regular bacteriological technique proved most satisfactory. The small amount of calcium hypochlorite solution introduced with the transferred material had no apparent deleterious effect, while the film of solution aids in preventing contamination during transfer.

For culture chambers, square screw-cap bottles with aluminum metal caps proved superior to other containers tried, such as petri dishes, covered watch glasses, and test tubes. When cultures are maintained for 8 to 24 months, there is less likelihood for the media to dry than with cotton plugged test tubes, and less opportunity for contamination as in the growing of an organism through a cotton plug. Tests with and without caps screwed tight have shown no disadvantage from tight closure, and have the advantage of less contamination over a period of months. For large embryos, as the peach, 1 ounce bottles are good; for smaller embryos, such as the cherry, pear, and apple, the $\frac{1}{2}$ ounce size is adequate.

For a considerable portion of the studies a culture solution has been used which consists of 0.6 per cent agar, 0.5 per cent glucose, and 0.15 per cent of the following salt mixture, designated T:

SALT MIXTURE T

10 gm. KCl 2.5 gm. $CaSO_4$ 2.5 gm. $MgSO_4$ 2.5 gm. $Ca_3(PO_4)_2$ 2.5 gm. $FePO_4$ 2 gm. KNO_3	Salts ground and mixed, and 1.5 gm. added to 6.5 gm. of agar and 5 gm. of glucose in 1 liter of water.

Since the medium has a pH of about 5.5, such a low concentration of agar should be either chilled in a refrigerator or otherwise rapidly cooled following autoclaving.

The salt may be kept ground and mixed in a stoppered bottle and used over a period of months as needed. There has seemed neither advantage nor disadvantage in filtering to remove any cloudiness.

In filling the bottles with medium, care has been exercised to place it in the bottom of the bottle by means of a pipette and otherwise to keep the sides and mouth of the bottle free from any medium. Sterilization has been at 15 pounds pressure for 20 minutes.

Unless otherwise indicated, cultures have been grown in daylight

in room temperature (*ca.* 18° C.). Using the technique described, peach embryos have been developed into small plants and maintained in a slowly growing condition in 1 ounce bottles for 17 months.

When plants have developed sufficient root and top growth, they may be shaken from the bottles, together with the agar medium, and planted in sterile quartz sand in 1½ inch pots until the plants are sufficiently large to shift to soil in larger pots. Young plants should be carefully shaded and gradually hardened.

AGAR CONCENTRATION.—Tests have been made with agar ranging from 0.5 to 10 per cent. WERKMEISTER (**26**) has reported good results with *Iris* seed cultured on 10 per cent agar, but not only has it been difficult to prepare so stiff a gel, but the behavior of similar embryos has been variable. Embryos which develop into plants soon suffer from lack of moisture. Both 1 and 2 per cent concentrations have proved useful, but best results have been secured with 0.6 per cent. At this concentration the agar is sufficiently stiff to support the embryo on the surface, yet availability of water does not prove a limiting factor. Lower concentrations, as 0.5 per cent, have proved difficult to use because of the ease with which they liquefy. La RUE's data, using a 0.75 per cent agar, agree closely with these results.

SALT CONCENTRATION.—A wide range of salt mixtures and concentrations have been used with similar results. These include that used by ROBBINS in root cultures and by WHITE in culturing root tips (**28, 29**); Knop's solution at dilutions used in sand cultures; and formula T, adapted from Crone's nitrogen-free formula.

Although concentrations have been varied from one to ten times a given strength, there has been no appreciable effect so long as the concentrations were not toxic to the plant. These results are in agreement with the previous findings by DIETRICH (**3**), TUKEY (**23**), and LA RUE (**11**), indicating a wide range of tolerance.

PH.—The general purpose formula used in these tests as formula T has a pH of about 5.5. Because the growth of embryos may alter the pH of the media, constant pH cultures were prepared after the formulae of ZINZADZE (**32**). These ranged from 3.8, 5.2, 6.0, 7.2, 8.2, and 8.6. Embryos grow to small plants on these media, with no

deleterious effect other than a slightly chlorotic appearance of leaves of plants grown for 70 days at pH 8.6, and a tendency for the media at 3.8 to liquefy in time owing to acid hydrolysis. The pH of these media remained unchanged throughout the experiment as tested at completion of the growth period.

ORGANIC AND GROWTH-PROMOTING SUBSTANCES.—The addition of various sugars to the media had an appreciable effect upon development, as will be later mentioned; but the chief responses from the addition of organic compounds, from temperatures, and from different photoperiods must be left to another paper.

The following organic compounds and growth-promoting substances have produced no consistent response in the manner and at the concentrations used:

Heteroauxin*
Indoleacetic acid 1 p.p.m.
Indoleacetic acid 1 p.p.m. and glycocoll 100 p.p.m.
Indoleacetic acid 10 p.p.m.
Indoleacetic acid 0.1 p.p.m.
Indolebutyric acid 1 p.p.m.
Indolebutyric acid 1 p.p.m. and glycocoll 700 p.p.m.
Indolepropionic 1 p.p.m.
Indolepropionic 10 p.p.m.
Propionic acid 1 p.p.m.
Propionic acid 10 p.p.m.
Yeast extract 200 p.p.m.
Yeast extract 10 p.p.m.
Yeast extract 10 p.p.m. and glycocoll 10 p.p.m.
Glycocoll 100 p.p.m.

* Supplied through the courtesy of Dr. F. W. WENT and Dr. K. V. THIMANN.

Growth of normal embryo

The developmental morphology of the embryo, seed, and fruit of the species to be studied has an important bearing upon the culture of excised embryos of deciduous fruits. Using the peach (**20**) as an example, growth of the carpel is in three stages: stage I, rapid development for 49 to 52 days after full bloom; stage II, retarded development from 5 to 42 days depending upon the variety; and stage III, rapid increase to maturity. During stage I the nucellus and integuments also make rapid increase, reaching maximum size by

the time stage II is reached, at which time the stony pericarp begins to form rapidly as sclerenchymatous tissue. The embryo during stage I develops slowly, in common with embryos of most angiosperms, remaining microscopic until the initiation of stage II. At that time it begins a rapid development and reaches maximum size in 12 to 28 days, depending upon the variety, followed by a period of accumulation of storage materials and internal change until maturity.

For other species the growth curves are characteristic. For the sweet cherry, stage I continues to 17 days following full bloom (**19**), for the sour cherry 21–22 days (**21**), and for the apricot 42 days (**14**).

Varieties of peach, cherry, and plum which produce early-ripening fruit fail to develop normally viable seed (**19, 20, 21**). Embryos of such varieties abort during stage II. The nucellus and integuments which have already reached maximum size collapse upon the partially developed embryo, to give the characteristic shriveled appearance of an abortive seed.

The apple and pear are similar in development but not identical. Growth of the fruit as measured by external measurements is not in the three well defined stages of the fruits of *Prunus*. The embryo grows similarly, however, in that there is delayed development following full bloom, followed in turn by a period of rapid embryo development. In the apple this rapid development begins 35 to 40 days (**15, 16**) after full bloom, while for the pear it is about 40 days (**15**).

Still another characteristic of seed of the deciduous fruits is the high energy content in the form of fat and the after-ripening period necessary for germination. Sweet cherry seed requires 16 weeks under moist conditions at 5° C. to complete after-ripening; peach seed 10 to 12 weeks; apple seed 6 to 8 weeks; and pear seed 4 to 6 weeks. These facts will be discussed more fully in relation to the results secured in culturing embryos at different stages in development.

Results with embryos of peach

The results show a definite and characteristic relation between the stage in development at which an embryo is excised and its subsequent behavior in culture. Embryos of the Elberta peach, received

from Athens, Georgia, at weekly intervals in nineteen lots from full bloom to fruit ripening, may be taken as representative, to which other samplings and other classes of fruits will later be referred. For clarity the results with one of several culture media will be considered, namely, that consisting of the nutrient agar given on page 633 with the addition of 10 p.p.m. of dried brewers' yeast. Likewise, unless otherwise noted, procedure A has been used, in which embryos have been treated with calcium hypochlorite solution (2 per cent chlorine) for 5 minutes. The stages in development of pericarp, nucellus and integuments, endosperm, and embryo, the technique used, and the performance of the embryo are given in connection with each sampling.

1. FULL BLOOM: FRUIT IN STAGE I, 3.4 MM. IN LENGTH; NUCELLUS AND INTEGUMENTS 0.9 MM.; EMBRYO NOT VISIBLE UNDER DISSECTING BINOCULARS.—The integuments were of waxlike consistency and could be chiseled cleanly from the nucellus by means of dissecting needles sharpened to a knife edge at the points. Embryos were too small to discern, but cultures made of the entire seed dissected aseptically in broth of medium T and cultured in liquid media on micro culture slides increased in length from 0.9 to 1.4 mm. in 9 days, as compared with an increase to 3.0 mm. on the tree.

2. SIX DAYS AFTER FULL BLOOM: FRUIT IN STAGE I, 7.1 MM. IN LENGTH; NUCELLUS AND INTEGUMENTS 2.7 MM.; EMBRYO NOT VISIBLE UNDER DISSECTING BINOCULARS.—Observations were similar to those with sample 1. Since embryos were too minute to be studied in culture no records of growth could be secured, but an entire seed removed aseptically was maintained in liquid medium T for 29 days with daily changes. The nucellus and integuments elongated slightly during this period.

3. THIRTEEN DAYS AFTER FULL BLOOM: FRUIT IN STAGE I, 10.0 MM. IN LENGTH; NUCELLUS AND INTEGUMENTS 4.2 MM.; EMBRYO NOT VISIBLE UNDER DISSECTING BINOCULARS.—The embryo sac could be removed intact, all but for the micropylar region, which remained firmly affixed to the nucellus and from which it could not be removed without rupturing. Embryos were still too minute to discern in culture. Entire seeds removed aseptically increased in liquid media T from 4.2 to 6.4 mm. in length in 3 days, a rate comparable to the in-

crease on the tree. In one instance two seeds were removed from a single fruit, attached to adjacent edges of the carpel. Cultured on the same slide the one increased from 3.5 to 6.2 mm. in length in 3 days, while the other made no increase.

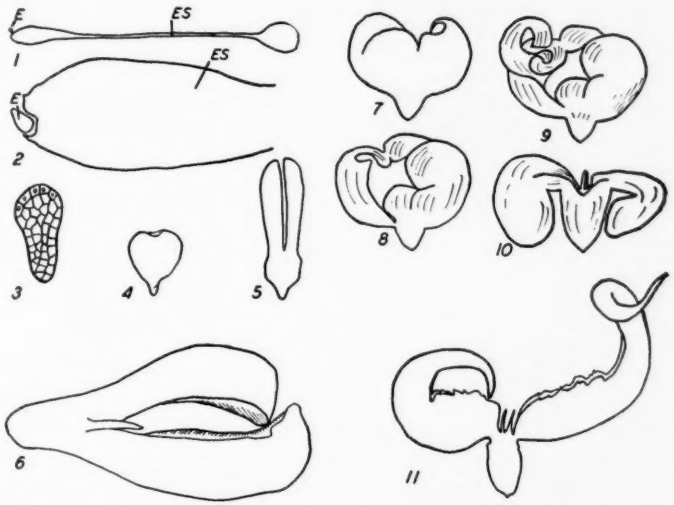
4. TWENTY DAYS AFTER FULL BLOOM: FRUIT IN STAGE I, 19.0 MM. IN LENGTH; NUCELLUS AND INTEGUMENTS 9.0 MM.; EMBRYO NOT VISIBLE UNDER DISSECTING BINOCULARS.—Using a disinfectant and culturing on agar media at this stage resulted in no increase in entire seed; and embryos were too small to be discerned.

5. TWENTY-SEVEN DAYS AFTER FULL BLOOM: FRUITS IN STAGE I, 25.8 MM. IN LENGTH; NUCELLUS AND INTEGUMENTS 10.5 MM.; CELLULAR ENDOSPERM 0.16 MM.; EMBRYO NOT VISIBLE UNDER DISSECTING BINOCULARS.—Entire seed treated with a disinfectant and placed on nutrient agar failed to increase in size, but upon dissection it was found that cellular endosperm had increased from 0.16 to 1.3 mm. in 13 days. Perhaps there may have been an increase in the size of the embryo as well, since embryo development follows endosperm development very closely (**20**), but the embryos were still too small to be observed in culture.

6. THIRTY-FOUR DAYS AFTER FULL BLOOM: FRUITS IN STAGE I, 34.0 MM. IN LENGTH; NUCELLUS AND INTEGUMENTS 13.6 MM.; EMBRYO SAC EXTENDING NEARLY TO CHALAZA; CELLULAR ENDOSPERM 1.6 MM. IN LENGTH; EMBRYO 0.16 MM.—The nucellus and integuments are pressed firmly against the ovary wall at this stage, and being tender and turgid are easily broken in opening the fruit. The best procedure was to split the fruit in halves along the ventral suture, the seed then being cut from the side of the carpel to which it was attached. The integuments could be stripped easily from the nucellus with needles and forceps, having passed from the softer, fleshier consistency of previous samples and not yet having reached the condition in which the integuments adhere to the nucellus and tear the nucellus with it. Furthermore, the nucellus was firm throughout and not yet digested by the endosperm.

It appears that the embryo is affixed to the micropylar end of the embryo sac, and the embryo sac in turn is affixed to the nucellus. The embryo sac could be lifted from the nucellus but not without rupturing at the micropylar end and exposing the embryo (figs. 1, 2).

It was necessary first carefully to chisel away the nucellus at the micropylar end, after which the embryo sac could be drawn out easily from the surrounding tissue. Isolated embryos failed to develop in culture following treatment with calcium hypochlorite solu-



FIGS. 1–11.—Results with peach embryos 34 days after full bloom (all drawings with camera lucida): Fig. 1, embryo sac (*es*) and embryo (*e*) dissected from immature seed 10.7 mm. long. Fig. 2, enlarged view of embryo and proximal end of embryo sac showing characteristic failure to remove embryo sac from nucellar tissue without rupturing; 1.0 mm. in length. Fig. 3, enlarged view of embryo, undifferentiated; 0.12 mm. in length. Fig. 4, differentiation of embryo in culture 10 days and increase from 0.12 mm. to 0.275 in length. Fig. 5, further differentiation of embryo 77 days in culture but abnormal; length 1.0 mm. Fig. 6, embryo cultured on agar within entire seed for 49 days, increasing from 0.32 mm. to 2.7 in length during that period. Figs. 7–10, anomalous growth of embryos in flowing media (7, 8, increases from 1.2 mm. to 3.8 in length and 4.6 mm. in width in 20 days; 9, same embryo after 166 days; 10, increases from 1.2 mm. in length to width of 2.6 mm. in 20 days). Fig. 11, 9-day development of embryo with cotyledons removed, excised from seed 61 days after full bloom; 9 mm. in breadth.

tion (2 per cent chlorine), although when cultured within the seed they differentiated and increased in size to 0.275 mm. in length in 10 days and 1.0 mm. in 77 days, as shown in figures 3–5. Treatment with calcium hypochlorite solution seemed harmful to tissues at this stage.

7. FORTY-ONE DAYS AFTER FULL BLOOM: FRUIT IN STAGE I, 40.5 MM. IN LENGTH; NUCELLUS AND INTEGUMENTS 17.1 MM.; CELLULAR ENDOSPERM 3.8 MM.; EMBRYO 0.32 MM.; COTYLEDONS DIFFERENTIATING.—Isolated embryos failed to develop in culture, although when entire seeds were cultured the embryo increased to 1.35 mm. in length in 8 days. In one instance the embryo reached 2.7 mm. in 49 days (fig. 5), with spreading and elongating cotyledons and general abnormal growth.

8. FORTY-EIGHT DAYS AFTER FULL BLOOM: FRUIT IN TRANSITION FROM STAGE I TO II, 44.5 MM. IN LENGTH; STONY PERICARP BEGINNING TO HARDEN; NUCELLUS AND INTEGUMENTS NEARLY MAXIMUM SIZE, 19.2 MM.; ENDOSPERM 8.0 MM.; EMBRYO ENTERING PERIOD OF RAPID INCREASE, 1.2 MM.; COTYLEDONS DIFFERENTIATING.—Isolated embryos treated with calcium hypochlorite solution failed to develop further in culture. Embryos dissected in broth of the medium and not treated with a disinfectant showed signs of development by a spreading of the cotyledons within 8 days, and increased from 0.9 to 3.8 mm. in length in 21 days, but failed to develop further. No chlorophyll was formed. In flowing media embryos developed into the anomalous forms shown in figures 7–10.

9. FIFTY-FIVE DAYS AFTER FULL BLOOM: FRUIT IN STAGE II, 46 MM. IN LENGTH; STONY PERICARP HARD; NUCELLUS AND INTEGUMENTS MAXIMUM SIZE, 20.5 MM.; ENDOSPERM 11.5 MM.; EMBRYOS IN PERIOD OF RAPID INCREASE, 2.0 TO 3.0 MM.—First indications of development following treatment with calcium hypochlorite solution were secured at this stage. In 48 hours after placing on agar the cotyledons had spread to 90 degrees with the central axis, while in 120 hours they had become recurved so that they touched at the tips below the hypocotyl. Chlorophyll formed in the dorsal surface tissue of the cotyledons, and the central axis of the epicotyl lengthened to 1 mm., but remained white throughout (fig. 12).

10. SIXTY-ONE DAYS AFTER FULL BLOOM: FRUIT IN STAGE II, 46 MM. IN LENGTH; STONY PERICARP HARD; NUCELLUS AND INTEGUMENTS MAXIMUM SIZE, 20.5 MM.; ENDOSPERM 16.5 MM.; EMBRYO INCREASING RAPIDLY, 5.4 MM.—Chlorophyll formed slowly, appearing first in the surface tissues of the dorsal side of the upper cotyledons. The lower cotyledon, in direct contact with the medium, then fre-

quently elongated 2 to 4 mm. and became spongy. Later the cotyledons spread at right angles to the central axis, followed by chlorophyll development on the ventral surfaces of the cotyledons. The central axis of the epicotyl lengthened to 1–2 mm., but remained white throughout, and the hypocotyl lengthened to 2–3 mm. In one instance, in which the cotyledons were excised by accident, the remaining portion of the embryo developed in 19 days to the anomalous form shown in figure 11.

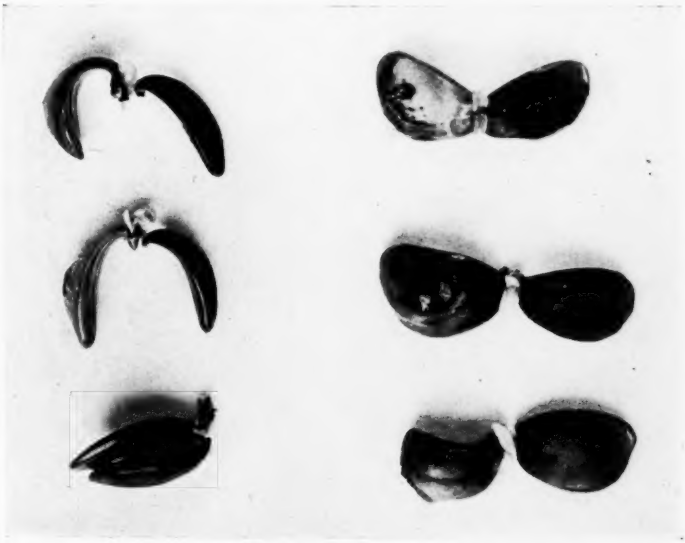


FIG. 12.—Development of embryos excised 51 days after full bloom, showing greening and spreading of cotyledons and white epicotyl.

11. SEVENTY-THREE DAYS AFTER FULL BLOOM: FRUIT IN STAGE II, 46 MM. IN LENGTH; STONY PERICARP HARD; NUCELLUS AND INTEGUMENTS MAXIMUM SIZE, 20.5 MM.; ENDOSPERM 17.8 MM.; EMBRYO INCREASING RAPIDLY, 16.4 MM.—Chlorophyll formed rapidly in the cotyledons, which spread at right angles to the central axis, the hypocotyl elongating 2 to 4 mm. with roots developing 10 to 15 mm. in length, and occasionally to 30 mm. The central axis of the epicotyl lengthened 1 to 2 mm. and became green. It terminated in a

rosette of six to ten small white appendages 0.5 to 1 mm. in length, much resembling stipules (fig. 13*A*). The appearance of the epicotyl was that which might be produced by the failure of the internodes of a central axis to elongate and upon which only the stipules had developed and in which no chlorophyll had formed.

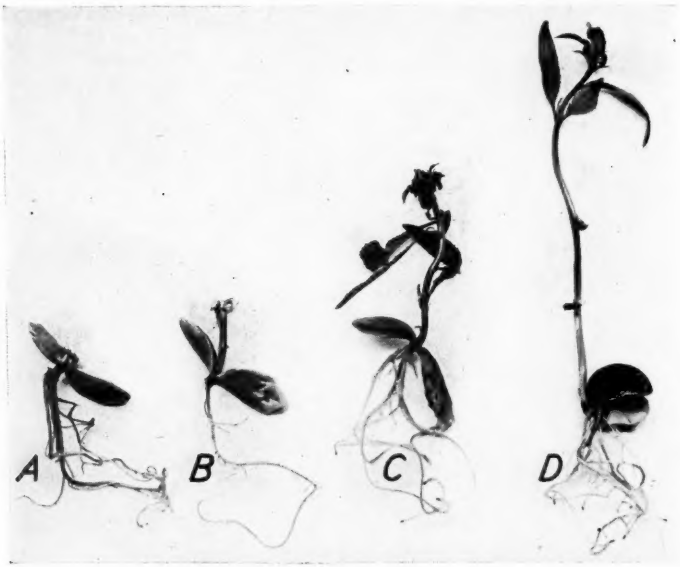


FIG. 13.—Typical growth patterns of peach seedlings developing from embryos excised from fruit at following ages after full bloom: *A*, 73 days; *B*, 80 days; *C*, 87 days; *D*, 94 days.

12. EIGHTY DAYS AFTER FULL BLOOM: FRUIT IN TRANSITION FROM STAGE II TO III, 48.0 MM. IN LENGTH; STONY PERICARP HARD; NUCELLUS AND INTEGUMENTS MAXIMUM SIZE, 20.5 MM.; ENDOSPERM NEARLY ALL DIGESTED; EMBRYO NEARLY MAXIMUM SIZE, 18.0 MM.—Chlorophyll formed on both dorsal and ventral surfaces of the cotyledons, which separated to 90 degrees with the central axis. The hypocotyl lengthened to 2–4 mm., frequently developing into roots 10 to 20 mm. in length, or even 30 mm. The central axis of the epicotyl lengthened to 7–15 mm. in 21 days and became green, being surmounted by a rosette of eight to twelve anomalous stipule-

like appendages 1 mm. in length, but without chlorophyll development (fig. 13*B*). Along the stem one or two green stipule-like appendages appeared in which chlorophyll developed. The general appearance was as of an elongation for an internode or two of the axis from the sample taken 73 days after full bloom, but with the internodes of the remainder of the stem still unelongated at the apex to give a rosette of white stipules.

13. EIGHTY-SEVEN DAYS AFTER FULL BLOOM: FRUIT ENTERING STAGE III, 50.0 MM. IN LENGTH; STONY PERICARP HARD; NUCELLUS AND INTEGUMENTS MAXIMUM SIZE, 20.5 MM.; ENDOSPERM NEARLY COMPLETELY DIGESTED; EMBRYO NEARLY MAXIMUM SIZE, 18.8 MM.—Chlorophyll formed on both dorsal and ventral surfaces of the cotyledons, which spread to 90 degrees with the central axis. Roots developed on all specimens and were 10 to 15 mm. in length in 28 days. The central axis of the epicotyl lengthened to 20–22 mm., green throughout, terminating in a rosette of stipule-like appendages 1 mm. in length in which chlorophyll had not developed. True leaves developed in addition to the stipules, although occasionally such leaves were broader than is typical of peach leaves and often had crinkled margins and whitish areas along the edges. The general appearance was as of a still further elongation of the internodes of the short axis from the preceding two samples, the further development of chlorophyll in the stipule-like appendages and the development of leaves (fig. 13*C*).

14. NINETY-FOUR DAYS AFTER FULL BLOOM: FRUITS IN STAGE III, 51 MM. IN LENGTH; STONY PERICARP HARD; NUCELLUS AND INTEGUMENTS MAXIMUM SIZE, 20.5 MM.; ENDOSPERM NEARLY ENTIRELY DIGESTED; EMBRYO MAXIMUM SIZE, 19.8 MM.—Chlorophyll formed on both the dorsal and ventral surfaces of the cotyledons, which separated at right angles with the central axis, but the chlorophyll became less abundant than in preceding stages. Roots developed vigorously to 25–30 mm. in length in 20 days. The central axis of the epicotyl elongated to 40 mm., terminated in a rosette of small, green stipule-like appendages 1 mm. in length. Occasionally one or two stipule-like appendages appeared along the stem. They were sometimes entirely green, sometimes white, and sometimes part green and part white. Occasionally also characteristic peach leaves

formed 25 to 35 mm. in length, yet these leaves were often broad and crinkled and had whitish areas along the margins. The general appearance was as of a still further elongation of the internodes of the short stem (fig. 13*D*).

15. ONE HUNDRED AND THREE DAYS AFTER FULL BLOOM: FRUIT IN STAGE III, 52.0 MM. IN LENGTH; STONY PERICARP HARD; NUCELLUS AND INTEGUMENTS MAXIMUM SIZE, 20.5 MM.; ENDOSPERM ALMOST ENTIRELY DIGESTED; EMBRYO MAXIMUM SIZE, 19.8 MM.—Only a small amount of chlorophyll was found in the cotyledons, which spread at right angles to the central axis. Roots developed vigorously and stem length reached 40 to 45 mm., terminated in a rosette of some whitish and some green leaflike appendages 1 mm. in length. Leaves formed along the stem but were frequently broad and crinkled with whitish areas along the margins. The nodes were still relatively close together, giving the appearance of a dwarfed plant (fig. 14*A*).

16. ONE HUNDRED AND EIGHT DAYS AFTER FULL BLOOM: FRUIT IN STAGE III, 59.0 MM. IN LENGTH, COLORING; STONY PERICARP HARD; NUCELLUS AND INTEGUMENTS MAXIMUM SIZE, 20.5 MM.; ENDOSPERM ALMOST ENTIRELY DIGESTED; EMBRYO MAXIMUM SIZE, 19.8 MM.—Roots developed vigorously and stems reached 45 to 50 mm. in length with green peachlike leaves. In 17 days the plants were transplanted from the culture bottles to soil. The leaves were often broader than typical peach leaves and the plants were dwarfish. The cotyledons developed no chlorophyll, but served as storage and nutritive organs, gradually becoming shriveled and dried (fig. 14*B*).

17. ONE HUNDRED AND EIGHTEEN DAYS AFTER FULL BLOOM: FRUIT IN STAGE III, 60.0 MM. IN LENGTH; STONY PERICARP HARD; NUCELLUS AND INTEGUMENTS MAXIMUM SIZE, 20.5 MM.; ENDOSPERM NEARLY ALL DIGESTED AWAY; EMBRYO MAXIMUM SIZE, 19.8 MM.—No chlorophyll found in the cotyledons at this stage. Vigorous root and shoot development occurred so that plants were transplanted to soil in 14 days. Growth was somewhat dwarfish (fig. 14*C*).

18. ONE HUNDRED AND TWENTY-TWO DAYS AFTER FULL BLOOM: FRUIT IN STAGE III, 63.0 MM. IN LENGTH; "HARD" RIPE; STONY PERICARP HARD; NUCELLUS AND INTEGUMENTS MAXIMUM SIZE, 20.5 MM.; ENDOSPERM NEARLY ALL DIGESTED AWAY; EMBRYO MAXIMUM SIZE, 19.8 MM.—Plants developed with vigorous root and shoot growth, al-

FIG. 14.—Typical growth patterns of peach seedlings developing from embryos excised from fruit at following ages after full bloom: *A*, 103 days; *B*, 108 days; *C*, 118 days. *D*, future development after placing in subdued light at 45° F. for 30 days.

though somewhat dwarfish. They were transplanted to soil in 14 days. No chlorophyll formed in the cotyledons.

19. ONE HUNDRED AND TWENTY-NINE DAYS AFTER FULL BLOOM: FRUIT IN STAGE III, 65.0 MM. IN LENGTH, SOFT RIPE; STONY PERICARP HARD; NUCELLUS AND INTEGUMENTS MAXIMUM SIZE, 20.5 MM.; INTEGUMENTS BROWN; ENDOSPERM NEARLY ALL DIGESTED AWAY; EMBRYO MAXIMUM SIZE, 19.8 MM.—Plants developed in 14 days which were only slightly dwarfish, with vigorous root and shoot development. They were transplanted into soil at the end of this time. Chlorophyll failed to develop and the cotyledons became somewhat shriveled, as though material had been utilized from them in the growth of the plant.

FUTURE DEVELOPMENT OF PLANTS

All plants failed to maintain an uninterrupted shoot growth beyond 50 to 70 mm., as contrasted with plants which develop from after-ripened peach seeds and reach a height of 300 to 400 mm. during the growing season. The cessation of terminal development and failure of the axis to elongate resulted in all cases in plants with at least some degree of dwarfing. Plants from embryos excised at the earliest stages were most dwarfed, and those from later stages least dwarfed. These last appeared similar to those described by FLEMION (5) from non-after-ripened embryos of mature peach seed.

When placed in the greenhouse in soil, the plants remained in this stage of arrested development, the stem became woody and some leaves abscissed as when plants enter a period of dormancy. They were then placed in a nursery cellar which provided subdued light and a temperature of about 45° F. In 30 days the plants were returned to the greenhouse. They immediately resumed rapid growth (fig. 14*D*), free from any abnormalities, such as characterized the earlier stages of development. Whether a shorter period of time would have accomplished the same results or what other factors might have brought about the same results is not known, since no other treatments were used. Close examination of the new shoot growth showed that it arose not from the terminal bud but from a lateral bud in the axil of one of the leaves near the tip of the shoot, as already described by DAVIDSON (2).

Subsequent behavior of the seedlings was similar to that of seedlings from after-ripened seeds. Planted into the orchard they grew into trees, some of which have borne fruit at 4 years of age. General fruit and tree characters were "normal," the only differences being the variations in individuals to be expected among seedling peaches.

The youngest embryos which were successfully cultured and grown into orchard plants were those excised 73 days after full bloom, and 56 days before the fruit was ripe. Considerable difficulty was experienced with damping-off fungi. Embryos 80 days old from full bloom were raised to orchard plants much more easily but still were difficult to handle because of the fungi. From 94 days to 129 days after full bloom the stand of seedlings was nearly perfect and little difficulty was experienced in growing the plants to orchard trees, some of which have fruited.

Results with other varieties of peaches during different seasons and from different sources

The embryos of the other four varieties of peaches received from Georgia behaved, in general, similarly in culture to the embryos of Elberta. A few points of difference are worth noting. First there was a spread of several days in blooming between the different varieties, in which Greensboro bloomed first and Elberta last. Since the rates of development of the fruit, seed, and embryo are nearly identical for all varieties, it follows that development of Greensboro was several days in advance of Elberta on the same calendar date. For example, 55 days after full bloom, embryos of Greensboro were about one-third maximum size; those of Carman, one-quarter; those of Hiley, one-quarter; those of Belle, one-quarter; and those of Elberta, one-sixth.

The response of the embryo in culture reflected the more advanced development of some varieties over others on the same date. For example, 61 days after full bloom the separation of the cotyledons and the development of chlorophyll was greatest in Greensboro, next greatest in Carman, and least in Elberta. Similarly, 73 days after full bloom, all the embryos of Greensboro and Carman had developed chlorophyll after 10 days in culture, as compared with one-third of

the embryos of Hiley, and one-fifth of those of Belle and Elberta. This relationship between varieties continued throughout the season.

Embryos of the early-ripening varieties aborted before they completely filled the integuments. Such embryos, although cultured at intervals for several weeks thereafter, developed only to the degree characteristic of embryos at the time of aborting. That is, embryos of Greensboro which had aborted 61 days after full bloom, and which were cultured 19 days later, developed in culture only to the stage of spreading the cotyledons and forming chlorophyll, as is characteristic of 61-day embryos. Embryos of Elberta, being non-abortive, behaved in culture on this date similar to 80-day embryos, roots frequently forming 5 to 20 mm. in length and the central axis of the epicotyl growing to 7 to 15 mm. in length.

The fact that the samples of peaches received from Georgia were several weeks further developed than those from Geneva, New York, and those from Geneva several days further than those from Youngstown, New York, gave an interesting comparison of embryos of the same variety cultured on the same day but in different stages of development. In all cases the embryos developed characteristic of the stage of development at which they were excised, so that strong shoot and root development appeared from embryos received from Georgia, whereas embryos from peaches from Geneva cultured on the same date developed only short shoot growth, and those from Youngstown developed only to the white rosette stage.

In addition to the varieties mentioned, embryos of the following thirty-one varieties, cultured during the seasons of 1932 to 1936, and secured from both Youngstown and Geneva, New York, representing a wide range of climatic and cultural conditions, seasonal development, and season of fruit ripening, gave substantially the same results: Alexander, Alexander Crosby, Arp, Belle, Blood Leaf, Canada, Carman, Champion, Chili, Crosby, Delicious, Eagle Beak, Early Crawford, Elberta, Foster, Golden Jubilee, Greensboro, Krummel, Lola, Mikado, Morellon, Mountain Rose, Rochester, Rosebud, St. John, Triumph, Troth, Valiant, Veteran, Waddell, and Ward Late.

Results with embryos of cherry, plum, apricot apple, and pear

The results in culturing excised embryos of the sweet cherry, sour cherry, plum, apricot, apple, and pear indicate the same general trend for these classes of fruits as for the peach, the species of *Prunus* following a little more closely than the apple and pear.

For a given species, the response of the embryos in culture is in accordance with the characteristic curve of growth of the embryo for that species. That is, the embryo of the sweet cherry begins its period of rapid development 17 days after full bloom; the sour cherry 21 days; the peach 49 days; the apricot 42 days; the apple 35–40 days; and the pear 40 days.

Accordingly, embryos of the sweet cherry responded in culture at an earlier calendar date than did embryos of the peach. When excised embryos of the peach had later reached the same stage of development as that of the cherry previously cultured, the growth in culture was similar.

Within a given species, embryos of the various varieties used behaved similarly, with the minor differences to be mentioned in the following paragraphs.

Apricot

Embryos of the variety of apricot used, Alexander, behaved similarly to those of the peach. They developed vigorously in culture and developed into strong plants.

Sweet and sour cherry

Embryos of the cherry responded more slowly in culture than embryos of the peach and the resulting plants were more delicate, less vigorous, and less easily handled. Using the embryo of the Mazzard cherry as the type, embryos excised prior to 27 days after full bloom, which had by that time reached a length of 1.48 mm., failed to develop in culture (fig. 15*A*). Beginning with embryos 30 days of age from full bloom, when the embryos had reached a length of 2.6 mm. compared with a maximum length of 7.2 mm. at maturity, the first indications of development were secured. The cotyledons of such embryos spread apart but developed no further. Embryos excised 32 days after full bloom, 3.1 mm. in length, developed

cotyledons which were thick and green, and two small white recurved leaves (fig. 15*B*).

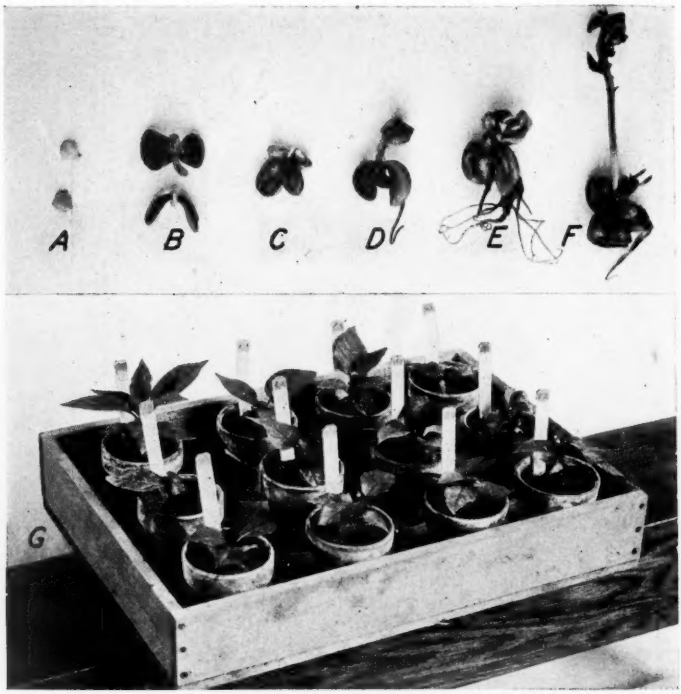


FIG. 15.—Growth in culture of sweet cherry embryos excised from fruit at various stages in development, time in days after full bloom: *A*, 27 days, no development in culture. *B*, 32 days, cotyledons spreading, thickened, and green, with two small white recurved leaves; *C*, 34 days, epicotyl terminated in rosette of small, white, stipule-like leaves; *D*, 36 days, hypocotyl thickened and elongated, roots developing, epicotyledonary axis elongating 2 to 4 mm., terminated in rosette of small, white, stipule-like leaves; *E*, 40 days, roots and small green leaves developing; *F*, 45 days, epicotyledonary axis elongated, leaves formed; *G*, 54 days, vigorous plant developments but with broad, crinkled, anomalous leaves and dwarfish habit of growth.

Embryos excised 34 days after full bloom, which were then 4.42 mm. in length, developed chlorophyll in the cotyledons in culture, the hypocotyl lengthened to 2–4 mm., and the epicotyl developed

as a rosette of small, stipule-like leaves in which no chlorophyll formed (fig. 15*C*). Thirty-six days after full bloom the embryos had reached a length of 5.8 mm. In culture they produced roots 3 to 5 mm. in length, developed chlorophyll in the cotyledons, and produced an epicotyledonary axis 2 to 4 mm. in length, surmounted by a rosette of stipule-like leaves in which chlorophyll was now developed (fig. 15*D*). Forty days after full bloom embryos had reached a length of 6.9 mm., which in culture developed roots and small green leaves (fig. 15*E*). Embryos excised 45 days after full bloom had reached maximum size, and developed in culture into plants with good root development, elongated epicotyledonary axis, and green leaves (fig. 15*F*).

Embryos excised 54 days after full bloom developed into vigorous plants, but which had broad, crinkled, anomalous leaves quite unlike a typical cherry leaf. Further, the habit of growth was dwarfish. As with the peach, such plants, placed at a temperature of 45° F. for 30 days in subdued light and then returned to the greenhouse, began a new shoot growth which no longer showed any anomalous growth forms or any dwarfish characters (fig. 15*G*).

Other varieties of sweet cherry used were Early Purple Guigne, Black Tartarian, Lyons, Seneca, Rockport, Kirtland, Burbank, Oswego, Yellow Spanish, Windsor, and Schmidt.

Sour cherries used were Early Richmond, English Morello, and Brusseler Braune. They behaved similarly to the sweet cherry. The embryo of the sour cherry, however, is less fully developed than that of the sweet cherry on the same date. Accordingly embryos of the sweet cherry in culture were developing into normal plants on the same day that embryos excised from the sour cherry were only beginning to develop chlorophyll in the cotyledons.

As with the peach, the earliest ripening varieties produced abortive embryos. The earliest, Early Purple Guigne, aborted so early that the embryos seldom reached the stage at which they developed in culture. Only occasionally was an embryo of the Early Purple Guigne found sufficiently advanced to develop.

An interesting observation in this connection was made with embryos of varieties ripening in succession and all cultured on the

same date, namely, Seneca, Burbank, Knight, and Black Tartarian. The embryos of the earliest ripening variety, Seneca, having aborted at a very early stage, developed only to the degree characteristic of that stage. Embryos of the next ripening variety, Burbank, having aborted at a slightly later stage, developed more fully in culture. Embryos of Knight, which ripens still later, developed still further in culture; and embryos of Black Tartarian, the latest ripening of the group, developed the furthest.

PLUM

Embryos of the varieties of the European plum Oullins, Italian Prune, and Middleburg responded in culture as well as, and similarly to, embryos of the peach. Of these three varieties, Oullins ripens early, Italian Prune in mid-season, and Middleburg late.

Unlike the varieties of peach, which all came into full bloom on the same date, thus giving a similar embryo development for all varieties on a given calendar date, Middleburg blossoms earliest, Oullins next, and Italian Prune last. On the same calendar date, therefore, embryos of Middleburg developed most fully in culture, Oullins next, and Italian Prune least, thus corresponding to the succession in bloom. But, computed on the basis of days following full bloom, embryos of all three developed in culture as characteristic of the interval following full bloom at which they were excised.

No development in culture was observed from embryos excised prior to 61 days from full bloom. Excised at the following intervals after full bloom, the characteristic development of embryos was as follows: 69 days, chlorophyll developed in the cotyledons; 72 days, hypocotyl elongated 2 to 4 mm. and epicotyledonary leaves recurved but remaining white; 78 days, roots developed and a rosette of small whitish stipule-like leaves formed; 83 days, epicotyledonary axis elongated to 2–5 mm. and the rosette of stipule-like leaves becoming green; 86 days, stem elongated to 15 mm.; 90 days, both root and shoot growth vigorous but elongated stem terminating in a rosette of green leaves; 98 days, vigorous plant development.

As in the peach, all plants were dwarfish, and when placed at a temperature of 45° F. in subdued light for 30 days, they began new and normal growth.

Embryos of the American varieties Tecumseh and DeSoto developed in similar stages, but the plants grew more feebly. Tecumseh came into full bloom a few days earlier than DeSoto, so that excised embryos of the former were always a few days in advance of those of the latter and behaved in culture as characteristic of that stage.

Apple and pear

Embryos of the apple and pear failed to develop as vigorously in culture as did those of the species of *Prunus*, particularly as those of the peach and apricot. The type of plant into which they developed was characteristic of the age from full bloom at which they were excised, and was similar to those already described for the peach. Shoot development was slender and weak, leaves were small, and roots were slender and lateral roots seldom developed.

The stage of development which embryos of the pear and apple reached on the tree at a given calendar date were sometimes variable, adding to the difficulty of an accurate interpretation of results. Unlike the peach, different varieties of which reach full bloom on nearly the same day, different varieties of apple and pear reach full bloom at successive intervals over a period of a week to 10 days, or sometimes longer. Furthermore, apple flowers are borne in a corymb and those of the pear in a cyme, so that there is a difference in time of bloom of individual flowers in the same cluster. Also, the flowers may be borne terminally, laterally, and on spurs—all on the same tree—and all varying slightly in the time at which they reach full bloom. Whitehouse (**30**) has shown that fruits which are of the same size in early season develop at the same rate. No doubt tagging of blossoms which bloom on the same day, or hand pollination, would result in more uniform material for culture.

Nevertheless the results were consistent with embryos of the same age. As in the case of the plum, embryos of varieties which reached full bloom earliest were furthest advanced on a given calendar date and responded in culture accordingly. McIntosh, for example, bloomed 6 days earlier than Rome Beauty. Embryos of McIntosh excised on the same day as embryos of Rome Beauty developed into plants more advanced in type. Yet embryos of Rome Beauty excised 6 days later developed into plants of similar type.

APPLE.—Apple varieties used were Red Astrachan, Early Harvest, Maiden Blush, R. I. Greening, McIntosh, and Rome, representing a wide range in season of ripening. Early Harvest embryos were consistently further developed the same number of days after bloom than embryos of the other varieties used. Since the cultivated apple is a combination of several species, this fact may account for the variation; or it may be that embryo development in varieties of apple is not so uniform as in the case of other fruits studied.

The other varieties of apple developed similarly. No development in culture was observed from embryos excised prior to 39 days from full bloom. Excised at the following intervals after full bloom, the characteristic development was as follows: 45 days, spreading of cotyledons and slight development of chlorophyll; 59 days, cotyledons dark green; 62 days, hypocotyl 2 to 4 mm. long, epicotyl developing into rosette of small, white leaves (fig. 16); 69 days, hypocotyl 6 mm. in length, epicotyl developing as rosette of small white and green leaves (fig. 16); 75 days, roots 10 mm. in length; 86 days, cotyledonary axis elongated to 4–6 mm., surmounted by small rosette of small white and green leaves, roots 20 to 25 mm. in length (fig. 16); 92 days, plants developed sufficiently to be grown in the greenhouse; 99 days, roots 25 mm. in length, epicotyledonary axis 8 mm. in length, surmounted by small rosette of green stipule-like leaves; 105 days, roots 25 to 30 mm. in length, stem 18 mm. with one or two slender leaves 6 mm. long; 120 days, normal but weak root, shoot, and leaf development; 134 days, stem 45 mm. in length, normal but weak shoot and root development.

It should be observed that no plants developed into normal seedling growth unless chlorophyll formed in the epicotyl. Chlorophyll development in the cotyledons and the formation of white leaves were not enough to carry the plants further.

PEAR.—Pear varieties used were Tyson, Seckel, Bartlett, and Kieffer, representing a wide range in season of fruit ripening. The first three are pure *Pyrus communis* L., the last is a hybrid between *P. communis* and *P. serotina*.

No development was observed in culture from pear embryos excised prior to 46 days from full bloom. Excised at the following

intervals after full bloom, the characteristic development was as follows: 66 days, cotyledons green; 69 days, cotyledons green, epicotyledonary axis 1 to 2 mm. in length, terminated in small rosette

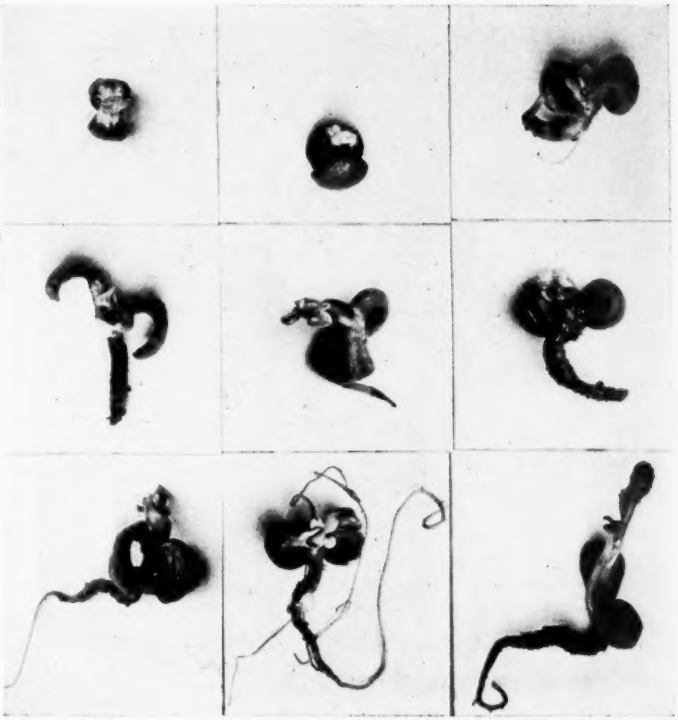


FIG. 16.—Growth in culture of apple embryos excised from fruit at various stages in development: Top row, 62 days after full bloom; cotyledons green, hypocotyl 2 to 4 mm. in length, epicotyl terminating in rosette of small, white leaves. Middle row, 69 days after full bloom; hypocotyl 6 mm. in length; epicotyl developing as rosette of small white and green leaves. Bottom row, 86 days after full bloom; epicotyledonary axis 4 to 6 mm. in length, surmounted by small rosette of small white and green leaves; roots 20 to 25 mm. in length.

of small white and green leaves (fig. 17*A*, *B*, *C*); 75 days, stem slender and 6 to 8 mm. in length, leaves slender and stipule-like, roots 22 mm. in length, plants developed sufficiently to be grown in the green-

house (fig. 17*D*, *E*, *F*, *G*); 81 days, stem slender and 18 mm. in length, leaves slender, roots 40 mm. in length; 97 days, stem weak and slender, 25 mm. in length, leaves normal but small and thin, roots 55 mm. in length (fig. 17*H*, *I*).

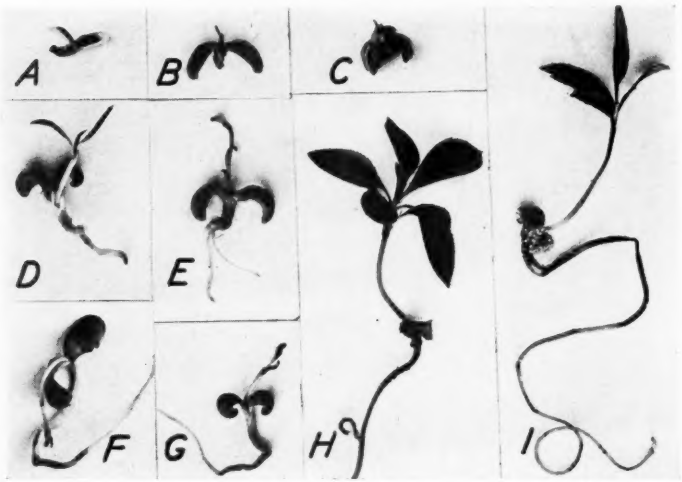


FIG. 17.—Growth in culture of pear embryos excised from fruit at various stages in development: *A*, *B*, *C*, 69 days after full bloom; cotyledons green, epicotyledonary axis terminated in small rosette of small white and green leaves; *D*, *E*, *F*, *G*, 75 days after full bloom; stems slender, leaves slender, roots 40 mm. in length. *H*, *I*, 97 days after full bloom; stem weak and slender, leaves normal but small and thin, roots 55 mm. in length.

Results without use of disinfecting agent

While the use of a disinfecting agent lends itself well to large scale methods of embryo culture, the question is immediately raised as to what effect such an agent may have upon the embryos. Of several materials used, namely, zonite, bichloride of mercury, and calcium hypochlorite, the last has proved very close to ideal. It is easily prepared according to the formula of WILSON (**31**) and as used by KNUDSON (**10**) for cultures of orchid embryos, giving almost exactly 20,000 p.p.m. of chlorine (2 per cent). It is inexpensive and easily handled, and immersion of embryos for 5 minutes has given almost perfect freedom from contamination.

On the other hand, it has been fairly simple to dissect embryos under aseptic conditions and to place them in culture without the use of a disinfecting agent. With the use of a transfer room, contamination has been reduced to 1 or 2 per cent. DAVIDSON (2) has found a transfer case helpful, and good results may be secured in the open laboratory provided proper precautions are taken. Both

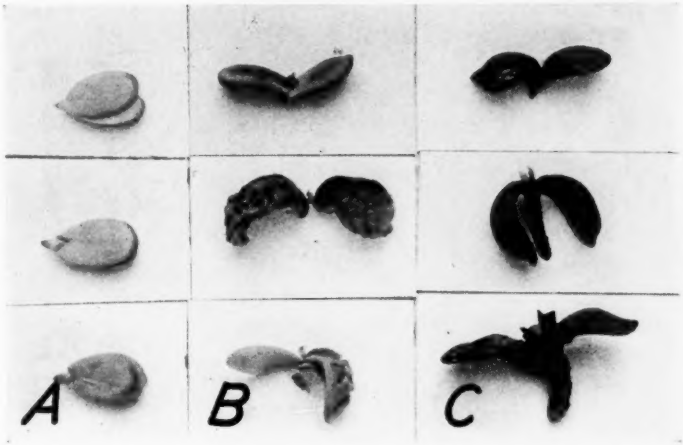


FIG. 18.—Effect of glucose upon apple embryos excised at early stages, showing that glucose is essential to chlorophyll formation and that higher concentrations are best for embryo development at early stages: *A*, no glucose in medium, no development; *B*, 0.5 per cent glucose, partial development; *C*, 2 per cent glucose, most development. *Cf.* fig. 19.

aseptic and disinfectant methods have been found practical and useful, depending upon the material used and the objectives desired.

The behavior of embryos placed in culture without treatment with a disinfecting agent differed in only minor points from the general behavior of embryos treated with calcium hypochlorite, as described in preceding paragraphs. Embryos excised at very early stages of development, 48 days after full bloom or earlier, failed to develop following treatment with calcium hypochlorite solution, although when embryos of this age were dissected in broth of the medium and then placed in culture, the cotyledons had spread

apart within 8 days and the embryos increased from a length of 0.9 mm. to 3.8 in 21 days. None developed into greenhouse plants.

With older embryos 51 to 94 days from full bloom, treatment with calcium hypochlorite delayed the formation of chlorophyll in the cotyledons 3 to 6 days, particularly on outer surfaces of the cotyledons, which were most exposed to the action of the material. In some instances, when embryo treatment was extended to 10 or 20

FIG. 19.—Effect of glucose upon cherry embryos excised at late stages, showing that glucose is inhibiting to the development of late stages: above, 2 per cent glucose in medium, poor growth; below, no glucose in medium, good growth. *Cf.* fig. 18.

minutes, chlorophyll failed entirely to develop on the dorsal surfaces of the cotyledons, although it later formed on the ventral surfaces after the cotyledons had spread apart.

With still older mature embryos, 100 days or more from full bloom, there seemed to be no effect upon development from treatment with calcium hypochlorite solution for 5 minutes.

In general calcium hypochlorite solution has been injurious to very young embryos, retarding to older embryos, and non-injurious to still older embryos.

Relation of medium to age of embryo when excised

The composition of the medium has affected variously the development of embryos excised at different stages. In the results that have been listed as the type in the present paper, the standard medium given in earlier paragraphs has been used. Varying several factors in the medium within the limits described, such as the pH and the composition and concentration of mineral nutrients, has had no appreciable effect upon embryo development. But the concentration of glucose has had a decided effect. In the case of early stages of embryo development, the presence of glucose in the medium is essential to chlorophyll development in the cotyledons. Further, although 0.5 per cent concentration of glucose favors development of both chlorophyll and the embryo, 2 per cent produces a still greater response. By the use of this higher concentration of glucose for embryos excised in early stages of development, still earlier stages can be successfully cultured.

By contrast, in late stages of embryo development, as when embryos have reached maturity, the presence of 2 per cent glucose in the medium is inhibiting. By the use of lower concentrations or by the elimination of glucose, still later stages may be successfully cultured.

By the use of 0.5 per cent glucose for the results presented in this paper, the range has been extended from early to late and has tended to give a more complete story than would otherwise have been the case.

Nevertheless the fact remains that by varying the concentration of glucose and by its absence from the medium, development of embryos has been altered. It is not too much to expect that with a more complete understanding of the chemical make-up of embryos at various stages in their development, and of the nutrients required, and with improved technique and methods of manipulation, still further progress may be made in altering or even eliminating some of the characteristic stages of development of embryos excised at various periods after full bloom and bringing them more nearly to what might be called normal plant development.

Discussion

The culturing in artificial media of immature embryos excised from growing plants has practical application to plant breeding and genetics, as the perpetuation of individuals in a given population which might otherwise fail to survive (**12, 18**). Further, it provides a method for studying the new sporophyte at earlier stages in development than that provided by the mature seed, and serves to focus attention upon the first expression of the new individual in the embryo rather than upon the plant developed from a mature embryo (**22**).

Throughout these studies, the stage of development of the embryo when excised from the fruit has overshadowed the other factors considered, but size alone is not a satisfactory criterion of whether or not an embryo can be grown successfully in culture. That chemical composition must be considered as well as size is shown by chemical analyses of developing peach embryos by TUKEY and LEE (**24**). They have shown that the peach embryo reaches nearly maximum size before any appreciable accumulation of fat (ether extract), nitrogen, and sugars begins. With embryos 17.5 mm. in length the content of fat was 0.40 per cent, of nitrogen 0.68 per cent, and of sucrose 1.06 per cent, whereas at maturity the embryos were only 1.5 mm. greater in length; but the content of fat was 30.67 per cent, of nitrogen 2.60 per cent, and of sucrose 2.32 per cent.

It would seem that a better comparison would be the age of the embryo on the basis of time, the differentiation of the embryo on the basis of morphological characters, or the development of the embryo on the basis of chemical composition.

That some of the youngest embryos did not respond to culture methods is not surprising, but that each age was expressed in a characteristic growth pattern is of particular interest. A similarity is at once recognized between the growth patterns presented in this paper and well known "juvenile" and "adult" forms in other plants. In discussing the differences in the formation of organs at different developmental stages, GOEBEL (**7**) emphasized the fact that all living things are in a condition of constant change, from earliest to latest stage in development. He cited differences in both function and in conformation of plant parts running through all plant phyla.

These facts emphasize that it is not the genetic make-up of an individual alone which is responsible for the expression of a plant in the formation and function of its various parts. As GOLDSCHMIDT (8) has explained, there are ". . . . two general notions in regard to the causal understanding of individual development. . . . . One is the notion . . . . that the action of the genes in controlling development is to be understood as working through the control of reactions of definite velocities, properly in tune with each other and thus guaranteeing the same event always to occur at the same time and at the same place. . . . . The second notion . . . . says that two types of differentiation are closely interwoven in the process of development, namely, independent and dependent differentiation. Independent differentiation means that a once started process of differentiation takes place within an organ or part of the embryo, even if completely isolated from the rest; dependent differentiation, however, requires the presence and influence of other parts of the embryo for orderly differentiation."

Of course it may be that the growth patterns described in this paper are merely the response of the plant to an unnatural and unfavorable environment. In a sense they may be considered maladjusted plants. The younger the embryo, the greater the difficulty in culturing and the greater the abnormal behavior. The older the embryo at the time it is excised from the mother plant, the more fully differentiated it has become and the less easily it is upset or thrown out of balance by an unnatural environment. The facts that a liquid medium is favorable at one stage of development and not at another, and that glucose is favorable at one stage and inhibiting at another, suggest that by providing a more suitable environment, by better technique, and by a better understanding of nutritional requirements, some of these growth patterns may be altered to more nearly the normal for the plant. On the other hand, the fact that even mature seeds must be after-ripened before they develop normally, suggests that there is an internal complex as well as an external environment which must be considered.

The failure of embryos to follow the pattern of embryonic development outside the environment of the mother plant raises the question as to what is the nature of the environment which brings

about "normal" embryo development on the plant. The shape of the embryo, it has been shown by HANNIG and others, as well as by these studies, is altered by its surrounding tissue. Does this mean that it is the shape of the campylotropous seed which physically causes a bean embryo to develop its curved shape? Or does it mean that some nutritional factors control the whole?

Finally, from the standpoint of developing mature plants from immature embryos, the facts reported in this paper point toward greatest success by maintaining an embryo in its natural environment or as nearly a natural environment as possible, so that it may follow the normal course of embryonic development before being subjected to a less favorable or less natural one.

Summary

1. Methods and results are given in culturing embryos of twelve varieties of sweet cherry (*Prunus avium*), five of sour cherry (*P. cerasus*), three of European plum (*P. domestica*), two of American plum (*P. americana*), thirty-two of peach (*P. persica*), one of apricot (*P. armeniaca*), five of apple (*Malus domestica*), and four of pear (*P. communis* and *P. communis* × *P. serotina*), during five growing seasons, 1932 to 1936 inclusive. More than 20,000 individual cultures have been made. Material has been cultured from Georgia and from three locations in New York State.

2. Embryos in culture do not pass through the embryonic stages characteristic of embryos on the mother plant. Instead they enter at once into an independent development characteristic of the age of the embryo when excised.

3. The growth pattern for peach embryos treated with a disinfectant and grown on 0.6 per cent agar media with 0.5 per cent glucose and salt mixture T may be summarized as follows: (A) no development earlier than 51 days of age after full bloom; (B) 51 days, spreading and greening of the cotyledons and small white epicotyledonary leaves; (C) 73 days, cotyledons green, hypocotyl 2 to 4 mm. and roots 10 to 15 mm. in length, central axis of epicotyl 1 to 2 mm. in length terminated by rosette of six to ten small white stipule-like appendages; 80 days, roots 10 to 20 mm. in length, central axis of epicotyl 7 to 15 mm. in length surmounted by rosette of

eight to twelve anomalous, white, stipule-like appendages; (D) 87 days, vigorous root development, central axis of epicotyl 20 to 22 mm. in length terminated by rosette of green stipule-like appendages; (E) 94 days, vigorous root development, central axis of epicotyl 25 to 30 mm. in length terminated by rosette of small, green stipule-like appendages, occasional peachlike but malformed leaves; (F) 105 days, vigorous root formation, stem 40 to 45 mm. in length terminated by rosette of small, green stipule-like leaves, with peachlike leaves along the stem; (G) 108 days, vigorous root and shoot development but leaves often broad and crinkled and plants dwarfish; (H) 118 days, vigorous root and shoot growth but dwarfish plants.

4. After 30 days in subdued light at 45° F., dwarfish plants began normal development and showed no further abnormal behavior.

5. Embryos of sour cherry, sweet cherry, apricot, plum, apple, and pear behaved similarly with minor differences.

6. Aseptic methods resulted in earlier response than when a disinfecting agent was used, but growth patterns were similar. Very young embryos were injured by a disinfectant.

7. Growth patterns were modified by altering the medium, especially glucose, in which at early stages of development glucose was beneficial and at later stages inhibiting.

8. The data are discussed with reference to physiological changes in the embryo itself, juvenile and adult forms of plants, and general problems of morphogenesis.

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GENEVA, NEW YORK

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CURRENT LITERATURE

Marine Algae of the Northeastern Coast of North America. By WILLIAM RANDOLPH TAYLOR. Illustrated by CHIN-CHIH JAO. University of Michigan Press, 1937. Pp. 427. Illustrated.

Botanists and others interested in the marine algae of the northeastern coast of this continent have long been at a disadvantage, because there has been no comprehensive treatment of them since FARLOW published his Marine Algae of New England, nearly fifty years ago. This need has been met in a very adequate manner by Professor TAYLOR's treatise covering the marine algae ranging from Virginia to the eastern American Arctic, including Hudson Bay. In large part the book presents the results of the author's study of living algae along the eastern shores of this country, especially in the region centering around Woods Hole, Massachusetts; his reexamination of the major phycological collections in this country, especially those of FARLOW and of COLLINS; and his study in European phycological herbaria, notably those of HARVEY and the younger AGARDH.

The book begins with an introductory account of the geographical distribution of marine algae within the area, and with a very useful sketch of the methods of collecting and preserving marine algae. The major portion is devoted to a systematic account of the algae. This is arranged in the conventional pattern, and with descriptions of orders, families, genera, and species, the last with habitat and geographical range indicated in some detail. There are also critical notes on many of the species. Keys are given for each order, family, and genus.

The descriptions of the various families and genera are noteworthy in that they incorporate modern studies on the life cycles and on the anatomy of reproductive organs. In the opinion of the reviewer the value of these descriptions would have been enhanced by citation of papers on anatomy and on life history studies, immediately following the descriptions of families and genera. As placed in the book these references are all listed according to species only, and intermingled with taxonomic references.

Most of the descriptions of the various species are original and give the author's concept of the limits of the species. In this he leans toward conservatism. He has also taken a conservative attitude on the distinctiveness and similarity of species among genera found on both American and European shores of the Atlantic.

A general key has been purposely omitted because the author thinks that, "In practice, the gross aspects of the common genera of marine algae are so readily learned that after a few days at the seashore the observer should be able to resort directly to the keys under the genera." In the opinion of the reviewer,

artificial keys, such as KYLIN has given for the Rhodophyceae of Puget Sound, are decidedly helpful to the beginning phycologist, to whom every alga is a systematic problem because there are no evident features showing its order or family. Dr. JAO's admirable illustrations compensate in the main for this lack of a key. These drawings include habit sketches of most of the genera and important details of anatomy of many of them, and are the best that have been published in the last fifty years.

American botanists are to be congratulated that the "standard book" on the marine algae of their Atlantic Coast has been done in so excellent a manner.—G. M. SMITH.

The Genus Bidens. Parts I and II. By EARL EDWARD SHERFF. Field Museum of Natural History, Bot. Ser. 16, 1937. Pp. 709. Illustrated.

The modest title of this publication scarcely indicates the scope of the work; it embodies the results of a comprehensive and critical study of the genus *Bidens*, a relatively large group of plants and a conspicuous and wide-spread element of the world's flora. As the author states, "The genus *Bidens* was so closely intertwined in botanical literature with *Coreopsis*, *Cosmos*, *Coreocarpus*, *Megalodonta*, *Dahlia*, *Isostigma*, *Heterosperma*, *Thelesperma*, and certain other genera of Compositae, that it became necessary in many cases to make a truly monographic study of these allied genera before attempting to progress further in the treatment of *Bidens* itself."

The preface contains a long list of institutions which have furnished material for study and of individuals who have co-operated in one way or another with the author in the course of his work. This fact, combined with the widely circulated preliminary publications on the group, gives ample evidence of the general interest aroused as well as an indication of the need for a comprehensive, uniform, and single taxonomic treatment of the group of plants concerned. A historical survey of the genus is followed by a section including morphology, histology, cytology, ecology, etc.

The main thesis is concerned with taxonomy. Fourteen sections of *Bidens* are enumerated and defined. Two hundred and thirty-five species, about 120 varieties, several forms, and a few hybrids are recognized as valid taxonomic entities. About 650 scientific names in various combinations are reduced to synonymy. The valid species are grouped for convenience into five geographical categories, as follows: Plants native to islands of Central Pacific Ocean; Plants growing in North and Central America and in the West Indies; Plants of South America; Plants of the Eastern Hemisphere, excluding Africa; Plants of Africa.

Under each of these geographical categories there is a dichotomous key, based on the more evident morphological characters leading to the species. Full and lucid descriptions are given of the species, varieties, and forms. The type specimen of each taxonomic unit, as well as the herbarium in which it is deposited, is indicated. Collections are copiously cited. Pertinent notes and comments are appended. The numerous illustrations, which have been faithfully

and carefully delineated by the author from types or authentic specimens, greatly aid in visualizing the characters recorded in descriptions.

A list of about 100 names to be excluded from *Bidens*, descriptions of two new species, a few names of doubtful status, and an index of collectors cited conclude the monograph.

This work is a distinct contribution to the literature of botanical science, and will constitute an authoritative reference work for many years to come.—J. M. GREENMAN.

A Textbook of Plant Virus Diseases. By KENNETH M. SMITH. Philadelphia: Blakiston, 1937. Pp. 615. Illustrated.

Further evidence that the field of phytovirology is coming of age as a science is the fact that plant viruses have been worked sufficiently to permit their classification and the writing of an excellent textbook on plant virus diseases, with classification of the viruses as its principle of organization.

Chapters 1–7 deal with viruses, beginning with Delphinium virus 1 and ending with Oryza virus 2. So far as possible (attained most completely for Nicotiana virus 1, and other Solanum viruses) the discussion of each virus deals with its physical and chemical properties, methods of transmission, differential hosts, diseases caused by it (arranged according to host families) including histopathology, and strains for each of which the preceding presentation is carried out as far as possible.

Chapter 8 deals with insects, etc., concerned in transmission of plant viruses and chapter 9 with suspected virus diseases requiring further investigation. An appendix in tabular form gives the names (scientific and common) of the host plants affected, symptoms induced, and the name of the disease incited by each virus. There are addenda, a general index, an index of viruses, and one of authors. Literature is cited at the close of each chapter. The illustrations are abundant and high grade.

This excellent book will be welcomed, not only by teacher and pupil, but also by the large number of researchers who are "discovering" viruses with appalling frequency, and the practical men who are attempting to stem the tide of virus creation.—G. K. K. LINK.

Introduction to Plant Pathology. By F. D. HEALD. New York: McGraw-Hill, 1937. Pp. 578. Illustrated.

This book is more simply written and covers less material than its excellent predecessor, HEALD'S "Manual of plant diseases." As a result it is more usable by elementary students but also less desirable for advanced investigators. It is characterized by the meticulous workmanship, accuracy, and clarity of the larger book, and will be welcomed by teachers of plant pathology and those seeking an introduction to the subject.

It is unfortunate that the author did not retain the order of presentation used in the Manual. In that work HEALD followed the pedagogically and theo-

retically sound principle of presenting first the non-parasitically induced diseases, and then the infectious diseases. This treatment might loosen the hold of the germ theory of disease as the cardinal principle in the experimental procedure of American and English plant pathologists. By reversing the order, especially in an introduction to the subject, the author elevates infection, which is only a special case of the class injury, to the position of central or leading concept, and degrades phytopathology to the position of only one phase of parasitology. A further lapse into, or a concession to, the characteristic conception and practice of phytopathology as a mycological discipline is evident in the fact that discussion of fungus induced diseases, with which the presentation of diseases is begun, is preceded by a chapter on the relation of fungi and bacteria to human affairs, in which considerable space is devoted not only to harmful relations in processed food stuffs, cheese, butter, leather, fabrics, and in animals, but also to useful relations of fungi.

The book, like its predecessor, designates plant pathologists "plant doctors," "medicine men of agriculture." Are these happy choices of terms? Those practicing the art of plant or crop protection may be designated plant doctors, if one must use such a label, but what about those engaged in investigations which are not oriented toward control, but toward advance of our knowledge about the basic biologic problems of injury and reaction to injury? By adopting this narrow conception of the role of the phytopathologist and implanting it in the minds of beginners who will be the leaders of the next generation, is not American phytopathology in danger of selling its birthright of a basic biologic science, rich with socially significant ideas, for the mess of pottage of a narrowly delineated art?—G. K. K. LINK.

Selected Topics in Colloid Chemistry. By ROSS AIKEN GORTNER. Ithaca, New York: Cornell University Press, 1937. Pp. xiii+169. Illustrated. $2.50.

During the first semester of the year 1935–36, the author filled the George Baker Fisher Non-Resident Lectureship in Chemistry at Cornell University. These lectures are now presented to the public in the form of a very attractive book. The first lecture, which serves as an introduction, bears the title Scientific Genealogy. It gives an intimate personal glimpse of the author's contacts with his teachers, an example of the spiritual genealogy of all scientists.

The chemical lectures are eight in number, with titles as follows: The beginnings of science; what is colloid chemistry; some basic concepts; some fundamental properties of colloidal systems; electrokinetics; surface tension, surface energy, interfacial tension, and molecular orientation; adsorption; and the water relations of biocolloids.

These lectures are informative and valuable expositions of the fields they cover, suitable material for all students of biology and biochemistry. They are not too technical, and would serve admirably as a foundation for the required understanding of colloidal behavior in physiological biology. GORTNER has

profited by the early contacts which he mentions in his first lecture; he is himself a great teacher and leader in his field. Many students will find help and inspiration in reading these excellent summaries of the progress of research and interpretation in the field of the colloidal state.—C. A. SHULL.

British Stem and Leaf Fungi (*Coelomycetes*). Vol. II. By W. B. GROVE. Cambridge: at the University Press. New York: The Macmillan Company, 1937. Pp. ix+406. $6.00.

This second volume completes the author's detailed morphological account of the British fungi belonging to the Coelomycetes. It includes those Sphaeropsidales comprising Sphaerioideae (with colored spores), Nectrioideae, Excipulaceae, Leptostromataceae, and the Melanconiales. The last order is divided into the Hyalospermae and the Phaeospermae.

As in volume I, most of the species treated have been seen and examined microscopically, and the descriptions of the various species are in the main purely morphological. A number of illustrations are given showing spore characteristics and the types of fructifications. There is included a short summary of geographical distribution, and there are indexes (1) of the Ascomycetes to which the coelomycetous fungi have been assigned by various investigators, (2) of the host genera, and (3) of the binomial names of the fungi treated.

The author's aim has been "to set before the English-speaking reader, for the first time in his own language and so far as it is illustrated by the British species of this group, a panoramic view of the skilful structure erected by the inimitable genius of Saccardo some fifty years ago, to include them all in one scheme." This aim he appears to have accomplished in admirable fashion. To all mycologists and plant pathologists who deal with this group the two volumes should be of outstanding value.—J. M. BEAL.